IC/88/321
INTERNAL REPORT
(Limited distribution)

International Atomic Energy Agency
and
United Nations Educational Scientific and Cultural Organization

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS

A SYSTEMATIC REVIEW ON AROMATIC L-AMINO ACID DECARBOXYLASE (5-HYDROXYTRYPTOPHAN DECARBOXYLASE)

M. Khalilur Rahman *
International Centre for Theoretical Physics, Trieste, Italy

and

Toshiharu Nagatsu
Department of Biochemistry, Nagoya University School of Medicine,
65, Tsurumai-Cho, Showa-Ku, Nagoya-466, Japan.

MIRAMARE - TRIESTE
November 1988

* Permanent address: Department of Biochemistry, University of Dhaka, Dhaka-1000, Bangladesh.

ABSTRACT

Aromatic L-amino acid decarboxylase (AADC, EC. 4.1.1.28) with L-5-hydroxytryptophan as a substrate (also called L-5-hydroxytryptophan decarboxylase, 5-HTPDC) decarboxylates L-5-hydroxytryptophan to serotonin (5-HT), an important neurotransmitter that involved in the regulation of neuronal functions, behaviour and emotion of higher animals. As it is an important enzyme, many researchers are now working on its physiological functions and properties and also on its isolation, purification and characterization from mammalian tissues. But up to now no systematic review studies have been done on this enzyme. We made systematic studies on this enzyme in tissues and brains of rats, and human subjects. We also developed highly sensitive assay methods of the enzyme. This new method led us to discover the enzyme in the sera of various animals. We examined the developmental changes of 5-HTPDC in the sera of animals. We discovered an endogenous inhibitor of the enzyme in the monkey blood. The purifications of the enzyme were performed by us and other researches from the sera, brains, adrenals, liver and kidneys of mammals. These and other results of up to date research papers on 5-HTPDC have been reviewed in this paper.

1. INTRODUCTION

L-DOPA decarboxylase (DDC) [1] and L-5-hydroxytryptophan decarboxylase [2] have been discovered to be the enzymes responsible for the biosynthesis of catecholamines [dopamine (DA), noradrenaline and adrenaline] (Fig.1) and indoleamines [5-hydroxytrptamine (serotonin, 5-HT) and melatonin] (Fig.2) [2-11].

The products are important biochemically and pharmacologically, because these monoamines are important intercellular messengers, such as neuro transmitters and hormones and involved in the regulation of neuronal functions, behaviour and emotion of higher animals.

It had been suggested that DDC and 5-HTPDC were two distinct enzymes and the enzyme commission had assigned separate numbers for them. (EC. 4.1.1.28 for DDC and EC. 4.1.1.29 for 5-HTP decarboxylase). Later evidence, however, confirmed the hypothesis that a single enzyme acts on both substrates [8, 9]. It has been concluded that L-DOPA and L-5-HTP are decarboxylated by the same enzyme, aromatic L-amino acid decarboxylase (AADC, EC. 4.1.1.28). Still the presence of more than one decarboxylase for aromatic L-amino acids in different organs and brain regions has been suggested by many workers mainly based on their physiological studies [12-17]. The still unsolved question whether DA and 5-HT, the two important neurotransmitters, are produced by the single or separate enzymers can only be solved by isolating and purifying the enzymes from the various organs and tissues, including brain and blood of mammals.

Up to now several research groups described the purification and characterization of AADC to homogeneity from various tissues of many species, for example, pig, guinea-pigs, rat kidney, human pheochromocytoma, rat liver, micrococcus percitreus and bovine brain [8, 18-27]. As many researchers are working on these neurotransmitters and their enzymes, we made these up to date review studies on AADC with L-5-hydroxytryptophan (5-HTP) as substrate (also called 5-hydroxytryptophan decarboxylase, 5-HTPDC).

2. ASSAY METHODS FOR L-5-HYDROXYTRYPTOPHAN DECARBOXYLASE

Many assay procedures have been reported on the activity of AADC: spectrophotometric [28, 29] spectrofluorimetric [6], gas chromatographic [30] and radiometric [8, 31, 32]. Amongst these methods, the radiometric method using L- or DL-[1-^{14}C]-DOPA as substrate to measure $^{14}CO_2$ formed [8, 31] may be most widely used, since the method is simple or sensitive. However, as CO_2, not dopamine, is the product measured by this radio-assay, non-

enzymatic decarboxylation cannot be distinguished from enzymatic decarboxylation. We had established a highly sensitive and specific assay for AADC activity using L-DOPA as substrate and D-DOPA for the blank by HPLC-voltametry [33]. This method is more sensitive than radio-assay and can only measure the enzymatic decarboxylation of L-DOPA.

Since AADC forms not only dopamine from L-DOPA, but also 5-HT from L-5-HTP as substrate. We have also tried to establish a new assay for AADC using L-5-HTP as substrate by HPLC-voltametry [34]. This method is now-a-days a very widely used method. Some of the important points of this method are mentioned here:

Materials

L-5-HTP, D-5-HTP, 5-HT, pargyline HCl and N-methyldopamine were obtained from Sigma (St. Louis, MO, USA); pyridoxal phosphate was from Katayama Chemicals (Osaka, Japan); Amberlite CG-50 was from Rohm and Haas (Philadelphia, PA, USA). All other chemicals were of analytical grade.

Rats were killed by decapitation. Immediately after the decapitation cerebral cortex was dissected. Rat serum was also collected. Human cerebral cortex was also dissected at autopsy from patients without a history of neurological disorders. The brains were homogenized with 0.32 M sucrose solution (1 part tissue plus 9 parts 0.32 M sucrose solution) in a Potter glass homogenizer. Amberlite CG-50 (type, 1, 100-200 mesh) was activated by washing with 2 M HCl, 2 M NaOH and finally with water, equilibrated with 1 M potassium phosphate buffer (pH 6.5), and stored in the same buffer.

Experimental procedures

The standard incubation mixture for L-5-HTP decarboxylase contained (total volume 400 µl, final pH 8.3): 30 mM sodium phosphate buffer (pH 9.0), 0.01 mM pyridoxal phosphate, 1.0 mM L-5-HTP (or D-5-HTP for the blank), 0.1 mM pargyline HCl, and the enzyme. Incubation was done at 37°C for 20-120 min., and the reaction was stopped by adding 80 µl of 3 M trichloroacetic acid. After 10 min, 1.82 ml of water and 100 µl of 0.01 M HCl containing 100-500 pmol of N-methyldopamine as an internal standard were added, and the mixture was centrifuged at 1600 g for 10 min. The supernatant was passed through a column (packed volume 0.5 ml) of Amberlite CG-50 (Na^+) equilibrated with 0.1 M potassium phosphate buffer (pH 6.5). The resin was washed twice with 4.5 ml of the buffer and with 200 µl of 1 M HCl. The 5-HT adsorbed was eluted with 1.4 ml of 1 M HCl. A 100-µl aliquot (or 50 µl) of the eluate was injected into the high-performace liquid chromatograph (Yanaco L-2000) with a Yanaco VMD-101 voltametric detector and a Yanapak ODS-T reversed-phase column (particle size

10 μm, 25 cm x 0.4 cm I.D.)(Yanagimoto, Kyoto, Japan). The carrier buffer for the liquid chromatography was 0.1 M potassium phosphate buffer containing 10% methanol, pH 3.2, with a flow-rate of 0.5 ml/min. The detector potential was set at 0.8 V against the Ag/AgCl electrode. The peak height of 5-HT was measured and converted to pmol from the peak height of N-methyldopamine added as an internal standard. The retention times under these conditions were: N-methyldopamine, 5.0 min.; 5-HT, 9.25 min.; and 5-HTP, 10.0 min.

This HPLC-voltametry system for the measurement of 5-HT and 5-HTP was found to be very sensitive. The standard curves of 5-HT and N-methyldopamine (internal standard) showed linearity from 200 fmol to 70 pmol. Among the brain regions of rats, cerebral cortex has the lowest enzyme activity. Therefore, for the development of this method, rat cerebral cortex homogenate was used as the enzyme source. Fig.3 shows the chromatographic pattern of the 5-HTPDC activity in rat cerebral cortex.

Since 5-HTP decarboxylase activity was found to be very low in human brains and only a small amount of data is available [35], we used this method for the measurement of the enzyme activity in the human cerebral cortex, which has the lowest enzyme activity. As shown in Table 1, 5-HTP decarboxylase activity in human brains was variable and very low. Rat cerebral vortex had about 40-fold higher activity than human cerebral cortex. The enzyme activity was discovered in rat serum by our method.

The present assay has many advantages. Firstly, it is very sensitive. The limit of sensitivity was about 1 pmol of 5-HT formed enzymatically. This method can measure v_{max} with saturated substrate concentration. This method is even more sensitive than our HPLC-voltametry method using L-DOPA [33] because the reaction with L-5-HTP proceeds linearly for longer time (2 h) than that with L-DOPA. The sensitivity of the present AADC assay is determined solely by the blank value using D-5-HTP as substrate. The blank is derived either from 5-HT formed by the non-enzymatic decarboxylation or from 5-HT contained in a crude enzyme preparation. Secondly, this method is specific, because it only measures enzymatically formed 5-HT from L-5-HTP. Thirdly, it is economical. Also the maintenance of the glassy carbon electrode of the electrochemical detector is easy. Fourthly, as N-methyldopamine is used as internal standard in each incubation mixture, this method is very accurate.

It should be noted that AADC activity was for the first time found in rat serum by the present method. This method is considered to be useful to measure AADC activity using L-5-HTP as substrate and a small amount of brain nucleus as enzyme source. Also, the assay of AADC in serum by

this method would be useful for physiological and pathological studies of aromatic amino acid metabolism.

3. L-5-HYDROXYFXYPTOPHAN DECARBOXYLASE ACTIVITY IN CENTRAL AND PERIPHERAL TISSUES AND SERUM OF RATS

There had been little separate data available for the activities of DDC and 5-HTPDC in different tissues. Sims et al. [36] measured the distribution of activities of DDC and 5-HTPDC in some brain regions of rats. Bourchard and Roberge [37] studied some properties and kinetic parameters of DDC and 5-HTPDC of cats. These and other results of some workers [38, 39] led us to make a systematic study of 5-HTPDC in seventeen different tissues (peripheral and brain tissues) and a study of some kinetic parameters of the enzyme in the pineal, adrenal and liver of rats [13].

We used fluorescence and HPLC assays for the measurement of the activity. Tissue distribution of 5-HTPDC activity in seventeen tissues and brain regions of rats is shown in Table 2. Pineal glands had the highest activity, followed by liver, kidney, adrenal and caudate nucleus. In brain regions, the activity in the caudate nucleus was about eleven times higher than that in the cerebellum. Since AADC has been reported to be particulate-bound in the brain [40], the effect of solubilization on the enzyme acitivity was examined. Sonication of the homogenate did not affect 5-HTPDC or DDC activity in pineal gland, adrenal glands and liver.

5-HTP decarboxylase activity obtained by fluorescence assay agreed well with the values obtained by HPLC-voltametry. For instance, we obtained the adrenal gland activities of this enzyme as 43 and 44.2 nmol/min/g wet weight of tissue by fluorescence and HPLC assays, respectively. To compare the properties of 5-HTPDC in different tissues, Km values, for 5-HTPDC in rat pineal gland, adrenal glands and liver were determined as shown in Table 3.

5-HTPDC activity was detected in rat serum by our new and highly sensitive assay for 5-HTPDC. The significance of serum 5-HTPDC activity in physiological and pathological processes remains to be investigated further.

The salivary gland had no activity due to the presence of the inhibitors [41, 42]. In our studies, addition of salivary gland homogenate to homogenates from other tissues completely inhibited AADC activity.

4. DETERMINATION OF L-5-HYDROXYTRYPTOPHAN DECARBOXYLASE IN SERUM OF VARIOUS ANIMALS

Although 5-HTPDC is distributed in various animal tissues, no data for the activity of this enzyme in serum is known as the enzyme activity in serum is very low. We had established very sensitive and specific assay methods for the activity of this enzyme using high performance liquid chromolography with electrochemical detection. L-5-HTPDC activity has been discovered in serum of rats, guinea-pigs, monkeys and mice by this method. The results are shown in Table 4 [43].

5-HTPDC activity in the serum of various animals were found to be widely variable. Serum from guinea-pigs had the highest activity, followed by the serum from rats, monkeys and mice. Chromatograms by HPLC-ED in the assay of 5-HTPDC activity in guinea-pig serum are shown in Fig.4 [43]. Formation of 5-HT was observed only with L-5-HTP as substrates. In Fig.4(D) 5-HT peak was due to endogenous serum 5-HT. Formation of 650 pmol of 5-HT for 60 min. at 37°C were calculated from the charts.

Using guinea-pig serum as enzyme for L-5-HTP as substrate, the optimum pH and pyridoxal phosphate concentration were found to be 8.4 and 0.01-0.10 mM, respectively. Reaction time-courses for 5-HTPDC of guinea-pig serum at 37°C was found to be linear up to 120 min. These results are similar to that of 5-HTPDC in other tissues. In human serum AADC activity is very low [44]. But we could measure the activity by using our sensity methods.

Kinetic studies on 5-HTPDC in guinea-pig serum also showed that its Km value (1.3×10^{-5}M) was almost similar to that of L-5-HTPDC in other tissues.

This is not the first enzyme in the catecholamine pathway that was found in serum. Dopamine β-hydroxylase (DBH) had been found in the sera from various mammals [45, 46] and it may be derived from the sympathetic nerve terminals and from the adrenal medulla [47]. Although the origin of serum 5-HTPDC is not clear, the serum enzyme activity with L-5-HTP as substrate may be useful for physiological and pharmacological studies.

5. DEVELOPMENT CHANGES IN RAT SERUM AROMATIC L-AMINO ACID DECARBOXYLASE WITH L-5-HYDROXYTRYPTOPHAN AS SUBSTRATE

Considering the question on the presence of a single or two AADC in mammals, we made a systematic study of DDC and 5-HTPDC activities in central and peripheral tissues of rats [13], by using our newly established assay methods based on HPLC with electrochemical detection [33, 14]. We

also discovered DDC, and 5-HTPDC activities in sera of rats, mice, monkeys and human [43, 44]. These previous results led us to examine the developmental changes of DDC and 5-HTPDC in sera of rats at different ages [14].

Changes in the activities [units (pmol/min/ml serum), shown in parentheses] of DDC and 5-HTPDC of rat serum and in body weight during development are shown in Fig.5. DDC activity was found to increase from birth (33 units) to 2 weeks of age (66 units), the decreased up to the age of 9 weeks (36 units), and again increased rapidly and reached the maximum at the age of 15 weeks (168 units). 5-HTPDC activity was lower than DDC activity at any ages during development. The activity was 7 units in new-born rats, decreased during 1 and 2 weeks (3 units), then increased rapidly from 2 to 3 weeks (12 units) and then decreased gradually to 7 units up to the age of 9 weeks. After 9 weeks, it increased to the maximum at the age of 15 weeks (37 units).

As shown in Fig.5, DDC and 5-HTPDC did not follow the same pattern as body weight. Both DDC and 5-HTPDC activity had a peak at about 3 weeks of age and a maximum adult level at 15 weeks of age. DBH activity also had been found to have a sharp peak at 2-3 weeks of age [48, 49]. It is known that serum DBH is derived mostly from the terminals of the sympathetic nerves, and the peak of serum DBH may represent rapid maturation of sympathetic nerves during this early post-natal period in rats. The peak of serum DDC and 5-HTPDC at 3 weeks of age may also represent the release of this enzyme from the terminals of sympathetic nerves.

In the study, serum DDC and 5-HTPDC activities showed similar, but significantly different developmental patterns. The serum enzyme at 2 weeks of age preferentially decarboxylated DOPA, thus the ratio of activity of DDC to 5-HTPDC was high, as compared to the enzyme at 15 weeks of age. Not only the Vmax but also the Km values for DOPA and 5-HTP as substrates were also different between the serum enzyme at 2 weeks and 15 weeks of age (Table 5). These results may suggest that the properties of rat serum enzyme are different at 2 weeks and 15 weeks of age, and that the origin of the serum enzyme may be different at 2 weeks and 15 weeks.

The origin of the sharp increase in AADC to the adult level from 9 weeks to 15 weeks of age is not clear. AADC is rich in liver, and since many serum enzymes are known to be derived from liver, one possible source of serum AADC in adult rats could be liver.

The developmental changes and properties of DDC were found to be different from that of 5-HTPDC in rat sera. This led us to study the developmental changes of AADC in serum of Japanese monkeys, Macaca fuscata fuscata, and the sera of the Japanese monkeys in all stages of their life have been found to contain an endogenous inhibitor of AADC which inhibits 5-HTPDC

activity of the monkey serum completely, but the activity of DDC only partially [50]. When we treated the monkey serum by DEAE-Sephacel chromotography, both DDC and 5-HTPDC activities were detected [50].

6. 5-HYDROXYTRYPTOPHAN DECARBOXYLASE ACTIVITY IN HUMAN BRAINS AND TISSUES

i) Demonstration of L-5-HTP decarboxylase in human brains

In human brains the activity of AADC is very low [35] and until now only some data on DDC activity in human brain are known [31, 33, 51, 52, 53, 54]. All reports [5, 7] indicate that 5-HTPDC activity may exist in human brain but is very low in comparison with DDC activity and was very difficult to measure. Vogel et al. [51] reported that 5-HTPDC activity could not be detected in the human brain areas and concluded that if the activity was present it was below the sensitivity of the method used. Reports at the 4th International Catecholamine Symposium [35] showed some data on human brain DDC but no data on human brain 5-HTPDC. Therefore, we used our sensitive methods [33, 34] to demonstrate 5-HTPDC activities and its properties in human brains [15]. The presence of 5-HTPDC in human brains was demonstrated by Lloyd and Hornykiewicz in 1972 [31], but almost no data for the properties of human brain 5-HTPDC are available because of its very low activity and availability of suitable method. We have applied our new method for the measurement of 5-HTPDC activity in human brains and found the method to be sensitive enough to detect the enzyme activity.

Chromatograms of HPLC-ED in the assay of 5-HTPDC activity in human caudate nucleus and hypothalamus are shown in Fig.6. Formation of 5-HT was observed only with L-5-HTP as substrate. In Fig.6, (A) and (B) show the activities in human caudate nucleus and hypothalamus, respectively.

Lineweaver-Burk plots illustrating the effect of the concentration of L-5-HTP on the rate of formation of 5-HT by human caudate nucleus as enzyme are shown in Fig.7. From the figure, the Michaelis constant (K_m) and the V_{max} values were calculated to be 90 ± 20 μM and 71 ± 46 pmol/min/g wet wt., respectively.

We found that human caudate nucleus and hypothalamus 5-HTPDC activities were 700 and 200 times lower than that of rat caudate nucleus and hypothalamus, respectively. We found higher activities in the hypothalamus than those in the caudate nucleus. In our studies on human caudate nucleus as enzyme the optimum pH and pyridoxal phosphate concentration for L-5-HTP as substrates were found to be 8.2 and 0.01-0.10 mM, respectively, which

were similar to those of other tissues of other animals, but the reaction time-course for L-5-HTPDC of this tissue at 37°C was found to be linear up to 240 min., which showed longer linearity of incubation time as compared with that for AADC in other tissues and serum. This is the first report describing the properties of human brain 5-HTPDC.

ii) L-5-Hydroxytryptophan decarboxylase activity in brains from normal human subjects and from patients with extrapyramidel diseases

L-5-Hydroxytryptophan decarboxylase in human brain has been reported to be even lower than the activity of DDC or almost undetectable [31, 51]. By assaying our highly sensitive HPLC-ED assay for 5-HTPDC activity [34] we have succeeded for the first time in measuring the enzyme activity accurately in various human brain regions [55].

5-HTPDC could be clearly demonstrated in almost all brain regions of controls and patients with extrapynamidal diseases as shown in Table 6. In the control brains 5-HTPDC activity was high in the hypothalamus, caudate nucleus, putamen and amygdala. In putamen, patients with Parkinson's disease, Shy-Drager syndrome, and perioral-dyskinesia had low activity than the control 5-HTP decarboxylase activity in putamen was not detectable in a case of Shy-Drager syndrome. Changes in AADC with L-DOPA or L-5-HTP as substrate varied in Parkinsonian brains [56].

iii) L-5-Hydroxytryptophan decarboxylase in human lung tissues: Comparison between normal lung and lung carcinomas

The enzyme activity in the human lung tissue has not been examined in detail. However, the enzyme activity was reported to be elevated in lung cancers such as the small cell carcinoma (SCC) of the lung [57, 58]. Therefore, Nagatsu et al. [59] have tried to measure the enzyme activity in the normal and cancer lungs tissues freshly obtained at surgey. SSC of the lung is a highly malignant human tumor which frequently produces some peptide hormones, such as vasopressin, adreno-corticotropin, calcitonin and gastrin-releasing peptide (or bombesin [60, 61] and has been proposed as belonging to the group of peptide hormone- and amine-synthesizing cells termed by Pearse as the amine precursor uptake decarboxylation (APUD) system [62]. According to this proposal, SCC of the lung was reported to have relatively higher activity of AADC than other lung tumor [57, 58].

5-HTP decarboxylase activity was measured in tumor sauptes with high DDC activity (Table 7).

Some SCC specimens contained relatively high concentrations of dopamine, but other SCC samples did not contain dopamine even though they had high DDC activity. In contrast, serotonin was detected in all seven

SCC specimens, and one each of ganglioneuroma and circinoid tumor, which showed high AADC activity. This was the first time that 5-HTPDC was found in SCC tissues containing high DDC for the first time.

It was also found that all SCC contain 5-HT, but that only a few SCC contain dopamine. One explanation for this result could be that SCC can produce or take up both dopamine and 5-HT, but can store preferentially 5-HT.

7. EFFECT OF ACTIVATOR/INHIBITOR ON L-5-HYDROXYTRYPTOPHAN DECARBOXYLASE

Aromatic L-amino acid decarboxylase requires pyridoxal phosphate (PLP) as the prosthelic group for its activity [63]. Some PLP is lightly bound to the apoenzyme as a Schiff's base, where another portion is purified dialyzable [12]. The purified enzyme of hog kidney contains 0.7-1.1 made of PLP per 112000 g of protein. The activity of the enzyme preparations is increased 2- to 5-fold on addition of PLP at 5×1^{-4}M [8]. However, up to now, no complete study on the effect of PLP deficiency on 5-HTPDC in central and peripherical tissues and serum of mammals has been available. It has been found that a decrease of PLP in the brain also decreases the α-Keloglutarate aminotransferase level [64, 65]. With the aim of making a detailed biochemical study of 5-HTPDC in mammals, we did a systematic study of the effect of PLP deficiency on the tissue distribution of this enzyme in fourteen different peripheral and brain tissues [17]. It had been shown that the in vivo administration of semicarbozide (SC) reduced the concentration of PLP in the brain which resulted in convulsion and that when the convulsions were prevented by simultaneous administration of pyridoxine, PL, or pyridoxamine, the PLP level rose to the normal level [66]. For our present study, therefore, the rats were treated with SC to produce PLP-deficiency. Each rat of the experimental group received a dose of 90 mg SC intraperitoneally. The solution of SC was prepared freshly in saline solution, and the dose was administered 80 min. before decapitation. The control group received a similar volume of saline alone.

As shown in Table 8, after SC-treatment, 5-HTPDC activity was decreased greatly in the kidney, adrenals, brain stem and heart ($P<0.001$); the liver and lung ($P<0.01$); and the cerebral cortex ($P<0.05$) of SC-treated rats. After addition of 10 μM PLP to the incubation mixtures, 5-HTPDC activity recovered significantly in the serum, caudate nucleus, brain stem, cerebral cortex and cerebellum of SC-treated rats. But in the adrenal glands

colliculi, lung, heart, liver and kidney, the activity was only partialy restored and was still low in comparison with that of control rats, even after addition of exogenous PLP in the incubation mixtures. The reason why the activities did not restore completely in some tissues, even after addition of PLP in the incubation mixtures, may be that the enzyme became unstable by SC-treatment, resulting in irreversible conformational changes in the enzyme molecule.

Siow and Dakshinamurti [67] had investigated the effect of pyridoxine deficiency on AADC using both L-DOPA and L-5-HTP as substrates in the rat brain. In pyridoxine deficiency, there were no parallel decreases in DDC and 5-HTPDC activities in various brain regions. Dialysis of brain homogenates, in the presence and absence of hydroxylamine, resulted in a total or near total loss of 5-HTPDC activity compared to DDC activity, indicating that PLP may be more tightly bound to DDC than to 5-HTPDC.

N-methyl-4-phenylpyridinium ion (MPP^+), a reaction product of a neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was found to inhibit AADC activity in rat clonal pheochromocytoma PC12h cells [68]. The enzyme activity was enhanced to several folds by addition of PLP, and MPP^+ inhibited the enhancement of the activity by exogenously added PLP. The inhibition was competitive to PLP, and the Ki value of MPP^+ was 26.7 ± 0.4 μM, while the Km value of PLP was 0.645 ± 0.053 μM. The inhibition was partly irreversible. The enzyme sample was incubated with MPP^+ and then dialyzed against phosphate buffer. After dialysis, the inhibited enzyme activity was only partly recovered by addition of PLP, even though MPP^+ was completely removed.

The compound MPTP has been reported to cause a Parkinson-like syndrome in humans following intravenous administration [69]. Now-a-days much attention has been focused on elucidating the mechanism of action of MPTP causing cell death. It has been shown that MPTP is oxidized to MPP^+ by monoamine oxidase (MAO) and also that the conversion of MPTP to MPP^+ can be blocked by MAO inhibitors. MPTP also reduced dopamine concentration in rat brain. However, the effect of MPTP on the enzyme catalyzing the formation of dopamine and 5-HT was not known until the effects of MPTP and MPP^+ were studied on AADC in rat brain [69]. The invitro effects of MPTP and MPP^+ on the activities of DDC and 5-HTPDC in rat brain are shown in Table 9.

In the presence of 5 or 10 μM MPTP or MPP^+, the activity of 5-HTPDC increased by about 70-107%. In constrast, the activity of the DDC was relatively unchanged. The increase in 5-HTPDC activity was in accordance with the increase in the brain level of serotonin following invivo administration of MPTP.

In course of our studies on the developmental changes of AADC in the serum of Japanese monkeys (Macaca fuscata fuscata), we found the presence of an endogenous inhibitor of AADC in all stages of monkey-life [70]. This inhibitor inhibited the serum 5-HTPDC activity completely, while DDC activity was partially inhibited. This inhibitor was non-dialyzable, but it could be removed from the monkey serum by DEAE-sephacel chromatography. After this treatment DDC and 5-HTPDC activities could be detected in the monkey serum.

Our report is not only a report on the presence of endogenous inhibitor of AADC in the tissues of animals; Dairman et al. [41] described a naturally occurring inhibitor of AADC in rat submandibular gland, first detected at about 6 weeks and Hashimoto et al. [42] described the partial purification and properties of DDC inhibitor in the same tissues of rats and found that the inhibitor is a protease. Before our finding of AADC inhibitor in serum, the presence of the endogenous inhibitor has been reported only in the salivary gland [41, 42].

There are several differences in the effect of inhibitors between DDC and 5-HTPDC. Chelating agents such as tetraethyllhiourea disulphide, diphenylthiocarbozone, ethylenediaminotetra-acetic acid (EDTAA) are inhibitors of 5-HTPDC. Hydroxylamine, norepinephrene, α-methyl-DOPA, α-methyl-m-tyrosine, DOPA also inhibit the decarboxylation of L-5-HTP. α-methyl-DOPA, α-methyl-5-HTP, N-methyl-DOPA hydrazine, α-methyl-DOPA hydrazine, NSD-1034, NSD-1015, and NSD-1055 are the inhibitors of AADC.

8. PURIFICATION AND CHARACTERIZATION OF AROMATIC L-AMINO ACID DECARBOXYLASE WITH 5-HTP AS SUBSTRATE

Several groups have desribed the purification of AADC to homogeneity from various tissues of many species: pig, guinea-pigs, rat kidneys [8, 18, 19, 20, 21], human pheochromocytoma [22, 23], rat liver [24], micrococcus percitreus [25] and bovine brain [26].

We found the presence of an endogenous inhibitor of AADC in the serum of the Japanese monkeys, Macaca fuscata fuscata, which preferentially inhibited 5-HTPDC activity. The inhibition was reversible and could be removed by ion-exchange chromatography and after this treatment 5-HTPDC activity could be detected in the monkey serum. We purified AADC using both L-DOPA and L-5-HTP as substrates [70] from sera of monkeys and rats. In serum AADC activities are very low. Therefore, we could not purify the enzyme to homogeniety (Table 10).

Christenson et al. [8] described the first purification of AADC to homogeniety for hog kidney and studied its properties, especially using both L-DOPA and L-5-HTP as substrates.

Ichinose et al. [23] purified AADC homogeneously and rapidly from human pheochromocytoma using HPLC. HPLC with gel permeation and hydrophobic columns was highly effective and the entire purification could be finished within 3 days (Table 11 and Fig.8).

Purified AADC showed a single band with an M_r of 50.000 on Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and decarboxylated L-DOPA, L-5-HTP, and L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS). Amino acid analaysis of purified AADC was also performed (Table 12).

The results concerning the subunit structure of AADC are controversial. Voltattorni et al. [71] reported that AADC from pig kidney is a heterodimer consisting of 43.000- and 50.000-Da subunits. On the other hand, Maneckjee and Bayling [22] reported that AADC from human pheochromocytoma is a homocliner with a 50.000-Da subunit from the study using a radiolabelled suicide substrate. The results of Ichinose et al. [23] also support that human pheochromocytoma AADC is a homoclimer of Mr 100.000 with a subunit of Mr 50.000.

Substrate specificity of the purified enzyme from human pheochromocytoma was examined with L-DOPA, L-5-HTP and L-threo-DOPS [23] as shown in Table 13.

Dominici et al. [24] purified AADC from rat-liver and discussed its properties in comparison with those of pig-kidney AADC.

Shirota and Fujisawa [21] purified AADC from rat kidney to homogeniety. The purified enzyme catalyzed L-DOPA, L-5-HTP, tyrosine, tryptophan and phenylalamine. To examine the identity of AADC from various tissues, they prepared a monoclonal antibody directed against the enzyme from rat kidney. Immunotitration and analysis by antibody-affinity chromatography followed by SDS-polyacrylamide gel electrophonesis revealed that the enzymes from the striatum, adrenal medulla, pineal gland, liver and kidney were indistinguishable with respect to immunological cross-reactivity and molecular size.

Albert et al. [27] reported that a single gene codes for AADC in both neuronal and non-neuronal tissues. Nishigaki et al. [26] purified AADC from bovine brain for the first time by affinity chromatography using a monoclonal antibody to the enzyme and it was compared with the AADC purified from bovine adrenal medulla by the same procedure.

A hybridoma clone secreting monoclonal antibody to AADC was established from a cell of a mouse immunized with highly purified AADC from bovine adrenal medulla. The monoclonal antibody was designated as NI-86, was IgG_1. Fig.9 shows a Western immunolot of partially purified AADC from bovine adrenal medulla. An essentially single band, with an apparent subunit Mr of 50.000 was observed in SDS/PAGE of the resultant enzyme from bovine brain. The results are shown in Fig.10. The purification procedures for AADC from bovine brain and adrenal medulla are summarized in Table 14. The results indicate that this purification procedure is useful for preparation of homogenous AADC on a large scale, which will assist further investigation of its chemical properties.

ACKNOWLEDGMENTS

One of the authors (M.K.R.) wishes to thank Professor A. Borsellino for his constant help, cooperation and suggestions during this work. He is thankful to Professors G. Ghirardi, G. Denardo and N.K. Rahman for their kindness and cooperation during his visit. He would also like to thank Professor Abdus Salam, the International Atomic Energy Agency and UNESCO for hospitality at the International Centre for Theoretical Physics, Trieste. He acknowledges the generous financial grant from the Swedish Agency for Research Cooperation with Developing Countries (SAREC).

REFERENCES

[1] P. Holtz, R. Heise and W. Spreyer, Nauryn-Schimiedeberg Arch. Exp. Path. Pharmak. 188, 580 (1938).

[2] C.T. Clark, H. Weissbach and S. Udenfriend, J. Biol. Chem. 210, 139 (1951).

[3] A. Yuwiler, E. Geller and S. Eiduson, Archs Biochem. Biophys. 80, 162 (1959).

[4] A. Yuwiler, E. Geller and S. Eiduson, Archs Biochem. Biophys. 89, 143 (1960).

[5] R. Kuntzman, A.S. Parkhurst, D. Bogdanski and B.B. Brodie, J. Neurochem. 6, 226 (1961).

[6] W. Lovenberg, H. Weissbach and S. Udenfriend, J. Biol. Chem. 237, 89 (1962).

[7] T.L. Sourkes, Pharmac. Rev. 18, 53 (1966).

[8] J.G. Christenson, W. Dairman and S. Udenfriend, Archs Biochem. Biophys. 141, 356 (1970).

[9] J.G. Christenson, W. Dairman and S. Udenfriend, Proc. Natn. Acad. Sci. USA 69, 343 (1972).

[10] G.A. Lancaster and T.L. Sourkes, Can. J. Biochem. 50, 791 (1972).

[11] W. Dairman, J.G. Christenson and S. Udenfriend, Life Sci. 13, 23 (1973).

[12] J. Awapara, R.P. Sandman and G. Hanley, Archs Biochem. Biophys. 98, 520 (1962).

[13] M.K. Rahman, T. Nagatsu and T. Kato, Biochem. Pharmacol. 30, 645 (1981).

[14] M.K. Rahman and T. Nagatsu, Biochem. Int. 3, No.6, 603 (1981).

[15] M.K. Rahman and T. Nagatsu, Neurochem. Int. 4, No.1, 1 (1982).

[16] M.K. Rahman, T. Nagatsu, I. Nagatsu, R. Tizuka and H. Narabayashi, Biomed. Res. 2, No.5, 560 (1981).

[17] M.K. Rahman, T. Nagatsu, T. Sakurai, S. Hori, M. Abe and M. Matsuda, Japan. J. Pharmacol. 32, 803 (1982).

[18] G.A. Lancaster and T.L. Sourkes, Can. J. Biochem. 50, 791 (1972).

[19] C.B. Voltattaori, A. Minelli, P. Vecchini, A. Fiori and C. Turano, Eur. J. Biochem. 93, 181 (1979).

[20] K. Strinivasan and J. Awapara, Biochem. Biophys. Acta 526, 597 (1978).

[21] K. Shirota and H. Fujisawa, J. Neurochem. (Plenum Press, New York, 1988) p.426.

[22] R. Maneckjee and S.B. Baylin, Biochem. 22, 6058 (1983).

[23] H. Ichinose, K. Kojima, A. Togari, Y. Kato, H. Parvez and T. Nagatsu, Anal. Biochem. 150, 404 (1985).

[24] P. Dominici, B. Tancini, D. Barra and C.B. Voltattorni, Eur. J. Biochem. 169, 209 (1987).

[25] H. Nakazawa, H. Kumagi and H. Yamada, Agric. Biol.Chem. 45, 9404 (1981).

[26] I. Nishigaki, H. Ichinose, K. Tamai and T. Nagatsu, Biochem. J. 252, 331 (1988).

[27] V.R. Albert, J.M. Allen and T.H. Joh, J. Biol. Chem. 262, No.19, 9404 (1987).

[28] V.E. Davis and J. Awapara, J. Biol. Chem. 235, 124 (1960).

[29] C. Streffer, Biochem. Biophys. Acta 139, 193 (1967).

[30] K.P. Wong and M. Sandler, Clin. Chim. Acta 50, 119 (1974).

[31] K.G. Lloyd and O. Hornykiewicz, J. Neurochem. 19, 1549 (1972).

[32] P. Laduron and F. Belpaire, Anal. Biochem. 26, 210 (1968).

[33] T. Nagatsu, T. Yamamoto and T. Kato, Anal. Biochem. 100, 160 (1979).

[34] M.K. Rahman, T. Nagatsu and T. Kato, J. Chromatigr. 221, 265 (1980).

[35] W. Sacks, W.H. Vogel, T. Nagatsu, K.G. Lloyd and M. Sandler, in Catecholamines: Basic and Clinical Frontiers, Eds. E. Usdin, I.J. Kopin and J. Barchas (Pergamon Press, Oxford, 1979) p. 127.

[36] K.L. Sims, G.A. Davis and F.E. Bloom, J. Neurochem. 20, 449 (1973).

[37] S. Bourchard and A.G. Roberge, Can. J. Biochem. 57, 1014 (1979).

[38] S. Udenfriend, Proceedings of Symposium on Chemical and Biological Aspects of Pyridoxal Catalysis, Rome, 1962, p.267 (Pergamon Press, Oxford).

[39] J.M. Saavedra, J. Neurochem. 26 585 (1976).

[40] G. Rodriguez, D.L. Amaiz and E. de Robertis, J. Neurochem. 11, 160 (1964).

[41] W. Dairman, O. Alingren and M. Marchelle, Fedn. Proc. 34, 777 (1975).

[42] S. Hashimoto, T. Ikeno, J. Hasegawa, T. Nagatsu and H. Kuzuya, Arch. Oral Biol. 25, 195 (1980).

[43] M.K. Rahman, T. Nagatsu and T. Kato, Life Sci. 28, 485 (1981).

[44] I. Nishigaki, H. Ichinose and T. Nagatsu, J. Clin. Biochem. Natri. 1, 181 (1986).

[45] R.M. Weinshilboum and J. Axelrod, Circ. Res. 28, 307 (1971).

[46] T. Nagatsu and S. Udenfriend, Clin. Chem. 18, 980 (1972).

[47] J. Axelrod, Pharmacol. Rev. 24, 233 (1972).

[48] T. Nagatsu, T. Kato, Y. Numata (Sudo), K. Ikuta, H. Umezawa, M. Matsuzaki and T. Takenchi, Nature 251, 630 (1974).

[49] H. Kuzuya, T. Ikeno, K. Ikeno, T. Kato and T. Nagatsu, Experientia 32, 16 (1976).

[50] M.K. Rahman, A. Togari, K. Kojima, K. Takahashi and T. Nagatsu, Mol. Cell. Biochem. 63, 53 (1984).

[51] W.H. Vogel, V. Orfei and B. Century, Pharmac. Expl. Therap. 165, 196 (1969).

[52] P.L. McGeer and E.G. McGeer, J. Neurochem. 26, 65 (1976).

[53] T. Nagatsu, T. Kato, Y. Numata (Sudo), K. Ikuta, M. Sano, I. Nagatsu, Y. Kondo, S. Inagaki, R. Iizuka, A. Hori and H. Narabayashi, Clin. Chem. Acta. 75, 221 (1977).

[54] T. Nagatsu, T. Kato, I. Nagatsu, Y. Kondo, S. Inagaki, R. Iizuka and H. Narabayashi, Adv. Neuro. 24, 283 (1979).

[55] M.K. Rahman, T. Nagatsu, I. Nagatsu, R. Iizuka and H. Narabayashi, Biomed. Res. 2, 560 (1981).

[56] T. Nagatsu, T. Yamaguchi, M.K. Rahman, J. Trocewicz, K. Oka, Y. Hirata, I. Nagatsu, H. Narabayashi, T. Kondo and R. Iizuka, Advanced Neurology, Eds. R.G. Hassler and J.F. Christ, (Raven Press, New York, 1984) p.40.

[57] S.B. Baylin, M.D. Abeloff, G. Goodwin, D.N. Carney and A.F. Gazdar, Cancer Res. 40 1990 (1980).

[58] S.B. Baylin, W.R. Weisburger, J.C. Eggleston, G. Mendelsohn, M.A. Beaver, M.D. Abeloff and D.S. Bttinger, N. Engl. J. Med. 299, 105 (1978).

[59] T. Nagatsu, H. Ichinose, K. Kojima, T. Kameya, J. Shimase, T. Kodama and Y. Shimosato, Biochem. Med. 34, 52 (1985).

[60] K. Abe, I. Adachi, S. Miyakawa, M. Tanaka, K. Yamaguchi, N. Tanaka, T. Kameya and Y. Shimosato, Cancer Res. 37, 4190 (1977).

[61] K. Yamaguchi, K. Abe, T. Kameya, I. Adachi, S. Tagauchi, K. Otsubo and N. Yanaihara, Cancer Res. 43, 3932 (1983).

[62] G.E. Pearse, J. Histochem. Cytochem. 17, 303 (1969).

[63] P. Holtz and F. Bachman, Naturwissenschaften 39, 89 (1952).

[64] R. Tapia, M. Dela Mora Péréz and G.M. Massieu, Ann. N.Y. Acad. Sci. 166, 257 (1969).

[65] F.N. Minard, J. Neurochem. 14, 681 (1967).

[66] T. Sakurai, T. Kuwahara and M. Matsuda, Jikeikai Med. J. 27, 13 (1980).

[67] L. Siow and K. Dakshinamurti, Exp. Brain Res. 59, 575 (1985).

[68] M. Naoi, T. Takahashi, H. Ichinose and T. Nagatsu, Biochem. Biophys. Res. Commun. 152, No.1 15 (1988).

[69] Short Communications, Biochem. Pharmacol. 35, No.15, 2640 (1986).

[70] M.K. Rahman, A. Togari, K. Kojima, K. Takahashi and T. Nagatsu, Mol. Cell. Biochem. 63, 53 (1984).

[71] C.B. Voltattorni, A. Minelli, C. Cirotto, D. Barra and C. Turano, Arch. Biochem. Biophys. 217, 58 (1982).

TABLE CAPTIONS

Table 1 Aromatic L-amino acid decarboxylase activity with L-5-HTP as substrate in human and rat cerebral cortex.

Table 2 Rat tissue distribution of AADC activity using L-DOPA and L-5-HTP as substrate.

Table 3 Kinetic parameters of AADC in pineal gland, adrenals and liver.*

Table 4 Aromatic L-amino acid decarboxylase activity with L-DOPA and L-5-HTP as substrates in serum of some animals.

Table 5 Km and Vmax values of aromatic L-amino acid decarboxylase in rat serum at 2 weeks and 15 weeks of age.

Table 6 Aromatic L-amino acid decarboxylase (AADC) activity with L-DOPA and L-5-Hydroxytryptophan (5-HTP) as substrates in brain regions of controls and Parkinsonian patients.

Table 7 5-Hydroxytryptophan decarboxylase activity and concentration of dopamine and sertonin in tumors with high DOPA decarboxylase activity.

Table 8 Effect of SC treatment on AADC activity in central and peripheral tissues and serum of rats.

Table 9 Effects of MPTP and MPP^+ on whole brain DOPA decarboxylase and 5-HTP decarboxylase activities in the rat.

Table 10 Partial purification of aromatic L-amino acid decarboxylase from rat and monkey sera.

Table 11 Purification of AADC from human pheochromocytoma.

Table 12 Amino acid composition of aromatic L-amino acid decarboxylase.

Table 13 Kinetic parameters of AADC from human pheochromocytoma for substrates.

Table 14 Purification of AADC from bovine brain (a) and adrenal medulla (b) by monoclonal-antibody affinity chromatography.

---

* Kinetic measurements were made as described in Materials and Methods. The pH values of incubation mixtures using L-DOPA and L-5-HTP as substrates were 7.2 and 8.3 respectively. The K_m values were calculated from Lineweaver-Burk plots [19], using Wilkinson's programme [20]. † Mean ± S.E.M., N = 7.

Table 1

Sample	AADC activity* (pmol/min/g wet tissue)	n
Rat cerebral cortex	3264 ± 53	4
Rat serum**	48.5 ± 9.4	3
Human cerebral cortex	89.3 ± 89.6	6
Range	21.2 – 246.0	

*The assay was carried out as described under Experimental procedures. Values are given as Mean ± S.E.M.
**pmol/min/ml of serum.

Table 2

Tissues	(mg wet wt)	AADC activity [nmoles·min^{-1}·(g wet wt tissue)$^{-1}$] L-DOPA as substrate	AADC activity [nmoles·min^{-1}·(g wet wt tissue)$^{-1}$] L-5-HTP as substrate	Ratio of activities L-DOPA / L-5-HTP
Pineal gland	0.125	1400 ± 139	227 ± 27	5.02 ± 0.24
		1.39 ± 0.13†	0.27 ± 0.02†	5.02 ± 0.24
Liver	2	444 ± 40	68.2 ± 6.9	6.5 ± 0.6
Kidney	2	418 ± 39	105 ± 8.0	3.99 ± 0.64
Adrenal glands	5	353 ± 27	43.5 ± 4.7	8.17 ± 0.68
		18.3 ± 0.2‡	2.1 ± 0.2‡	8.17 ± 0.68
Caudate nucleus	5	106.7 ± 10.5	21.2 ± 2.2	5.03 ± 0.17
Hypothalamus	1	71 ± 7	10.2 ± 1.2	7.83 ± 1.6
Colliculi	5	40.7 ± 4.1	5.1 ± 0.6	7.85 ± 0.38
Brain stem	10	24.7 ± 1.9	5.9 ± 1.1	4.46 ± 0.4
Small intestine	10	56.8 ± 10.6	8.0 ± 0.8	7.00 ± 0.85
Large intestine	10	44.8 ± 4.7	10.4 ± 1.2	4.37 ± 0.82
Lung	10	31.9 ± 4.3	4.16 ± 0.48	7.73 ± 0.77
Cerebral cortex	10	14.7 ± 1.1	1.52 ± 0.26	9.81 ± 1.71
Cerebellum	10	12.6 ± 1.7	1.87 ± 0.22	6.69 ± 0.70
Heart	10	11.9 ± 1.3	2.43 ± 0.11	5.02 ± 0.67
Spleen	10	4.9 ± 0.1	1.3 ± 0.03	4.29 ± 0.03
Blood serum	100	121 ± 11§	46.3 ± 7.7§	2.61 ± 0.15
Salivary gland	10	0.0‖	0.0‖	

* Each value is the mean ± S.E.M. for five rats, each homogenate being assyed in duplicate.
Activities for the two substrates were assayed simultaneously with the same tissue homogenate.
† Nmoles/min per pineal gland.
‡ NMoles/min per pair of adrenal glands.
§ Pmoles/min per ml of serum
‖ Not detectable.

Table 3

Tissues	K_m values† (μM)	
	L-DOPA as substrate	L-5-HTP as substrate
Pineal gland	32 ± 6	18 ± 6
Adrenal glands	102 ± 23	37 ± 5
Liver	160 ± 16	45 ± 6

Table 4

Samples	Aromatic L-Amino Acid Decarboxylase Activity[a] (pmol/min/ml of serum)		Activity with L-DOPA as substrate / Activity with L-5-HTP as substrate
	L-DOPA as substrate	L-5-HTP as substrate	
Guinea pig serum	349.0 ± 0.163 (4) (218.0-605.0)	177.0 ± 94.0 (5) (111.0-339.0)	2.0
Monkey serum	44.7 ± 19.7 (3) (22.0-57.7)	11.0 ± 5.7 (3) (4.4-15.3)	4.1
Rat serum	60.0 ± 26.5 (4) (36.4-82.4)	34.2 ± 9.1 (5) (26.2-45.1)	1.8
Mouse serum	20.8 ± 1.10 (4) (11.3-30.6)	10.0, 10.0 (2)	2.1

Numbers of samples and the ranges are shown in parentheses.
[a] The assay was done as described under Materials and Methods. The values are shown as MEAN ± S.E.M.

Table 5

Samples	Km (μmol)		Vmax (pmol/min/ml serum)	
	L-DOPA as substrate	L-5-HTP as substrate	L-DOPA as substrate	L-5-HTP as substrate
	(mean ± S.E.M.)			
2 weeks-old rat serum	237 ± 19	103 ± 30	70 ± 2	2.1 ± 0.2
15 weeks-old rat serum	41 ± 4	175 ± 15	217 ± 6	33.0 ± 1.0

Table 6

Brain region	AADC Activity (mean ± SE) L-DOPA as substrate (pmol/min/g tissue)		L-5HTP as substrate (pmol/min/g tissue)		Ratio of activity L-DOPA / L-5HTP
A. Controls					
Caudate nucleus (4)	226 ± 65	[88–384]	25.8 ± 7.1	[10–40]	8.8
Putamen (4)	184 ± 31	[102–384]	23.9 ± 5.8	[15–40]	7.7
Substantia nigra (1)	—		7.5		-
Hypothalamus (3)	685 ± 275	[140–1,020]	52.4 ± 24.5	[4.2–83.6]	13
Amygdala (3)	233 ± 183	[50–600]	9.5 ± 6.7	[3–23]	25
Reticular formation (1)	54.4		3.3		16
Raphé nucleus (2)	30.0	[20, 40]	7.5	[7.5, —]	4
Cerebral cortex (2)	60.0	[50, 70]	9.0	[8, 10]	6.6
Cerebellar nucleus (2)	35.0	[25, 45]	0.0	[0.0, 0.0]	—
B. Parkinson's disease					
Putamen (4)	111 ± 4*	[100–121]	7.2 ± 3.4*	[0.8–17.4]	15.4
Amygdala (2)	147	[86.5, 207]	212	[5.6, 418]	0.7
Reticular formation (1)	42.9		1.2		36
Raphé nucleus (1)	308		63.8		4.6
Cerebellar cortex (2)	124	[98.1, 149]	125	[27.7, 223]	1.0
C. Striato-nigral degeneration					
Caudate nucleus (2)	389	[495, 284]	58.1	[100, 16.2]	6.7
Putamen (1)	339		86.9		3.9
Mammillary body (1)	71.4		17.7		4.0
D. Shy-Drager syndrome					
Caudate nucleus (1)	114		1.8		63.0
Putamen (1)	8.1		0.0		-
E. Perioral dyskinesia					
Putamen (1)	16.2		9.4		1.7
Mammillary body (1)	0.0		3.8		

* $P < 0.05$ for difference between controls and Parkinson's disease. Numbers of samples are indicated in parentheses, and the individual activity in each patient in square brackets.

Table 7

	DOPA decarboxylase (pmole/min/mg protein)	5-Hydroxytrytophan decarboxylase (pmole/min/mg protein)	Dopamine (nmole/g tissue)	Serotonin (nmole/g tissue)
Small cell carcinoma	740	69.3	2.0	8.5
	1360	167	3.1	9.9
	1930	174	0.0	14.8
	2150	257	0.0	7.8
	66.7	3.1	0.0	10.0
	23.9	0.0	0.0	7.8
	780	74.4	0.0	6.9
Ganglioneuroma	888	71.4	2.4	6.2
Carcinoid tumor	1110	221	0.0	5.5

Table 8

Tissue	Amount of tissue used for assay (mg)	AADC activity (nmoles/min/g wet weight tissue) mean±S.E.M.			
		L-DOPA as substrate		L-5-HTP as substrate	
		With 10 μM PLP	Without PLP	With 10 μM PLP	Without PLP
A. Control					
Pineal gland	0.1	1424.4±215.3	213.0±29.6	192.7±81.9	44.4±28.3
Liver	4.0	597.0± 26.4	285.3±90.4	136.2±28.3	110.3±35.5
Kidney	4.0	428.0± 61.5	66.5± 9.2	57.6± 8.9	40.0± 4.6
Adrenal gland	4.0	207.6± 36.4	58.6± 8.8	36.1± 4.6	23.9± 4.5
Caudate nucleus	4.0	141.3± 12.0	32.2± 6.6	21.3± 5.1	13.8± 4.1
Hypothalamus	2.5	72.4± 20.0	18.3± 3.2	15.9± 1.8	11.8± 3.8
Colliculi	4.0	33.4± 7.3	17.1± 4.4	6.4± 1.5	3.9± 2.0
Brain stem	10.0	34.7± 6.2	19.5± 4.7	7.5± 1.2	5.7± 0.4
Cerebral cortex	10.0	19.7± 3.9	8.8± 2.0	3.9± 1.2	3.1± 0.7
Cerebellum	10.0	20.0± 6.5	12.1± 1.1	2.5± 0.8	1.4± 0.8
Lung	20.0	52.1± 6.1	16.3± 2.9	10.0± 1.2	6.4± 1.6
Heart	20.0	14.7± 3.7	4.5± 1.2	2.5± 0.2	1.7± 0.1
Spleen	20.0	4.4± 1.6	0.2± 0.1	1.7± 0.9	0.3± 0.2
Blood serum	100.0	27.3± 3.2§	2.8± 0.9§	9.6± 2.6§	3.6± 1.8§
B. SC treated					
Pineal gland	0.1	243.7± 45.2**	11.4±10.4***	47.4±22.9	0.6± 0.6
Liver	4.0	404.2± 64.3*	30.4± 4.4**	45.3± 7.6*	13.8± 7.6**
Kidney	4.0	216.0± 71.4	0.5± 0.05***	29.9± 6.3*	0.3± 0.2***
Adrenal gland	4.0	82.7± 22.7*	2.6± 2.4***	2.7± 0.7***	0.5± 0.2***
Caudate nucleus	4.0	61.1± 9.3**	20.5± 3.0*	23.8± 3.3	15.3± 4.0
Hypothalamus	2.5	39.0± 12.9	1.2± 0.2**	10.3± 3.8	6.9± 4.9
Colliculi	4.0	16.2± 3.4*	2.0± 0.7**	0.6± 0.6***	1.9± 1.1
Brain stem	10.0	20.6± 6.2	3.4± 0.7**	5.6± 1.2	1.5± 0.3***
Cerebral cortex	10.0	9.7± 3.8	0.7± 0.2**	2.0± 0.3	0.5± 0.4*
Cerebellum	10.0	6.7± 2.3	0.3± 0.1***	1.9± 0.7	0.1± 0.09
Lung	20.0	23.8± 4.0**	0.3± 0.1***	1.8± 0.4***	0.2± 0.1**
Heart	20.0	6.8± 1.8	0.2± 0.0*	1.0± 0.3**	0.1± 0.1***
Spleen	20.0	2.2± 0.3	0.8± 0.3	0.1± 0.05	0.1± 0.1
Blood serum	100.0	26.7± 5.0§	0.0± 0.0**§	11.5± 4.8§	3.2± 1.6§

***P<0.001, **P<0.01 and *P<0.05 as compared with the control. § pmoles/min/ml serum.

Table 9

Compound	Conc (μM)	DOPA decarboxylase activity*†	5-HTP decarboxylase activity*‡
None		986 ± 100	16.0 ± 3.4
MPTP	5	1089 ± 102 (+11%)	27.3 ± 6.0 (+71%)
	10	1097 ± 101 (+12%)	29.6 ± 6.0 (+86%)
MPP+	5	880 ± 57 (−9%)	31.6 ± 8.0 (+96%)
	10	909 ± 53 (−6%)	33.2 ± 4.6 (+107%)

* Brain homogenate was incubated at 37° for 15 min in a medium (1-ml vol.) containing: 80 μmoles sodium phosphate buffer (pH 6.7), 0.125 μmole pyridoxal 5'-phosphate, 10 μmoles 2-mercaptoethanol, 1 μmole DOPA (containing 0.1 μCi L-[3-^{14}C]DOPA) with and without MPTP or MPP+.

† Values represent mean ±S.E.M. for five separate experiments, each performed in duplicate. Enzyme activities are expressed in pmoles/min/mg protein. Numbers in parentheses show percent changes from control.

‡ Brain homogenate was incubated at 37° for 60 min in a medium (1-ml vol.) containing: 75 μmoles Tris buffer (pH 8.3), 0.3 μmole pyridoxal 5'-phosphate, 10 μmoles 2-mercaptoethanol, 0.6 μmole 5-HTP (containing 0.1 μCi DL-[3-^{14}C]5-HTP) with and without MPTP or MPP+.

Table 10

Fraction	Total volume (ml)	Total protein (mg)	Total activity (pmol/min)		Specific activity (pmol/min/mg protein)		Purification (- fold)		Yield (%)		Ratio DDC / L-5-HTPDC
			L-DOPA as substrate (DDC)	L-5-HTP as substrate (L-5-HTPDC)	DDC	L-5-HTPDC	DDC	L-5-HTPDC	DDC	L-5-HTPDC	
Rat serum	6.0	294	1260	420	4.31	1.42	1	1	100	100	3.0
$(NH_4)_2SO_4$, 25-55%	1.6	147	853	253	5.79	1.72	1.3	1.2	67	60	3.4
Bio-Gel A-1.5 m	9.8	46.1	815	311	17.7	6.7	4.1	4.7	64	74	2.6
DEAE-Sephacel I	8.6	7.22	668	321	92.4	44.6	21.4	31.2	53	77	2.1
DEAE-Sephacel II	9.2	1.35	483	149	356	110	82.6	78.0	38	35	3.2
Phenyl-Sepharose	2.6	0.14	113	22.0	805	157	187	111	8.8	5.2	5.1
$(NH_4)_2SO_4$, 0-80%	1.0	0.12	66.4	28.0	572	243	133	171	5.2	6.7	2.4
Sephadex G-150	4.0	0.034	38.8	16.6	1142	487	265	342	3.0	3.9	2.3
Monkey serum	34.0	2873	472	*	0.16	*	1	-	-	-	-
DEAE-Sephacel	67.0	322	1407	448	4.37	1.39	27.0	-		-	3.1
$(NH_4)_2SO_4$, 25-55%	2.3	37.0	464	353	12.3	9.35	75.0	-	-	-	1.3
Bio-Gel A-1.5 m	8.0	19.2	480	256	25.0	13.3	152	-	-	-	1.9
$(NH_4)_2SO_4$, 0-80%	1.9	13.7	414	268	30.3	19.6	184	-	-	-	1.5
Sephadex G-150	4.7	4.5	148	78	32.9	17.3	200	-	-	-	1.9

* Activity could not be detected due to the presence of endogenous inhibitor.
The purification was done as described under 'Materials and methods'.

Table 11

Steps	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (- fold)	Yield (%)
Homogenate	11000	44.8	0.004	1	100
100,000 g supernatant	8460	27.7	0.003	0.7	45
pH 4.7 supernatant	6930	12.5	0.002	40	98
DEAE-Sephacel	221	27.0	0.12	287	65
G-3000 SW	20.8	17.9	0.86	2860	45
Phenyl-5PW (1)	1.45	12.4	8.57	3430	33
Phenyl-5PW (2)	0.87	9.02	10.3		

Note U: μmol dopamine formed/min.

Table 12

Amino acid	Human pheochromocytoma (mol%)	Pig kidney[a] (mol%)
Aspartic acid[b]	7.3	6.3
Glutamic acid[b]	12.9	10.5
Serine	17.6	5.4
Glycine	15.3	8.8
Histidine	2.5	2.5
Arginine	3.0	5.7
Threonine	4.4	3.4
Alanine	9.4	10.8
Proline	3.7	4.9
Tyrosine	1.4	2.8
Valine	4.3	6.3
Methionine	2.8	2.3
Isoleucine	3.2	4.0
Leucine	6.0	12.3
Phenylalanine	2.9	5.4
Lysine	3.2	4.7
Cysteine	—[c]	2.1
Tryptophan	—[c]	1.8

Table 13

Substrate	K_m (M)	V_{max} (μmol/min/mg protein)
L-DOPA	4.6×10^{-5}	10.3
L-5-HTP	6.7×10^{-5}	1.55
L-*threo*-DOPS	1.3×10^{-3}	0.56

Table 14

Tissue	Step	Total activity (nmol/min)	Total protein (mg)	Specific activity (nmol/min per mg)	Purification (fold)	Yield (%)
(*a*) Brain	Homogenate	1001	11000	0.091	1	100
	100000 *g* supernatant	612	2556	0.239	2.63	61
	DEAE-Sephacel	519	302	1.72	18.9	52
	Monoclonal-antibody affinity chromatography	90	2.08	43.3	476	9.0
(*b*) Adrenal medulla	Homogenate	4177	3797	1.1	1	100
	100000 *g* supernatant	2782	1112	2.5	2.2	67
	DEAE-Sephacel	2548	72.8	35	31.8	61
	Monoclonal-antibody affinity chromatography	358	1.8	190	173	8.6

FIGURE CAPTIONS

Fig.1 Enzymatic synthesis of dopamine, noradrenaline and adrenaline.

Fig.2 Enzymatic synthesis of serotonin.

Fig.3 HPLC elution pattern of L-5-HTP decarboxylase incubation mixtures with homogenate of rat cerebral cortex as enzyme. The conditions were as described under Experimental procedures. The standard incubation mixture contained 5 mg of rat cerebral cortex. (A) Experimental incubation with L-5-HTP; (B) blank incubation with D-5-HTP; 250 pmol of N-methyldopamine (N-M-DA) were added to each sample after incubation. (C) Standard mixture of 50 µl, containing 17.5 pmol, each of L-5-HTP, 5-HT and N-M-DA.

Fig.4 HPLC eltuion pattern of the incubation mixtures for L-DOPA decarboxylase and L-5-HTP decarboxylase with guinea pig serum as enzyme. Standard incubation mixture contained 100 µl of serum. (A) and (B) are experimental and blank incubations with L-DOPA and D-DOPA, respectively. (C) and (D) are experimental and blank incubations with L-5-HTP and D-5-HTP, respectively.

Fig.5 Comparison of the body weights and developmental changes of L-DOPA decarboxylase and L-5-HTP decarboxylase activities in serum of rats at different age groups. •——• : L-DOPA decarboxylase, ▲——▲: L-5-HTP decarboxylase, ×—×: body weight, N.B.: new-born rats.

Fig.6 HPLC elution pattern of L-DOPA decarboxylase and L-5-HTP decarboxylase incubation mixtures with homogenates of human caudate nucleus and hypothalamus. The conditions were as described under Materials and Methods. (A) The standard incubation mixtures contained 33.3 mg and 20 mg of human caudate nucleus for the substrates L-DOPA and L-5-HTP, respectively. Incubations were done for 20 min. and 240 min. for the substrates DOPA and 5-HTP, respectively, at 37°C. 125pmol of dihydroxybenzylamine (DHBA) or 250 pmol of N-methyl-dopamine (N-M-DA) were added to each sample after incubation. (B) The standard incubation mixtures contained 20 mg and 8 mg of human hypothalamus for the substrates L-DOPA and L-5-HTP, respectively. Incubations were done for 20 min. and 180 min. for the substrates DOPA and 5-HTP, respectively, at 37°C. 250pmol of DHBA or N-M-DA were added to each sample after incubation.

Fig.7 Lineweaver-Burk plots illustrating the effect of the concentration of L-DOPA and L-5-HTP on the rate of formation of DA and 5-HT, respectively, by AADC from human caudate nucleus. The K_m and V_{max} values were calculated to be 414 ± 70 µM and 0.482 ± 0.054 nmol/min/g wet weight, for L-5-HTP decarboxylase, respectively, ×—×:L-DOPA as substrate; •——• : L-5-HTP as substrate.

Fig.8 SDS-PAGE. Lanes: (a) crude extract of human pheochromocytoma; (b)-(e) active fractions from chromatography of DEAE-Sephacel (b), G-3000 SW (c), first phenyl-5PW (d), and second phenyl-5PW (e); (f) and (g) standard proteins. These fractions were subjected to electrophoresis in 4-15% gradient polyacrylamide gel in the presence of SDS. The slab gel was stained for protein with Coomassie brilliant blue R-250. Approximately 1.8 µl of purified enzyme (e) was applied to the gel.

Fig.9 Western immunoblot of AADC partially purified from bovine adrenal medulla.

Standard proteins (a) and AADC [30-50%-satd.-$(NH_4)_2SO_4$ fraction] (b) were separated on SDS/polyacrylamide slab gels, transferred to a mitrocellulose sheet, and stained with Amido Black and monoclonal antibody to AADC from bovine adrenal medulla respectively. Standard proteins from top to bottom are: phosphorylase b(M_r 92500), bovine serum albumin (66200), ovalbumin (45000) and carbonic anhydrase (31000).

Fig.10 SDS/polyacrylamide-slab-gel electrophoresis of AADC from bovine brain purified by monoclonal-antibody affinity chromatography. (a) Standard proteins, from top to bottom: phosphorylase b(M_r 92500), bovine serum albumin (66200), ovalbumin (45000) and carbonic anhydrase (31000). (b) AADC preparation from a DEAE-Sephacel column, before loading on a monoclonal-antibody affianity column. (c) AADC from a monoclonal-antibody affinity column.

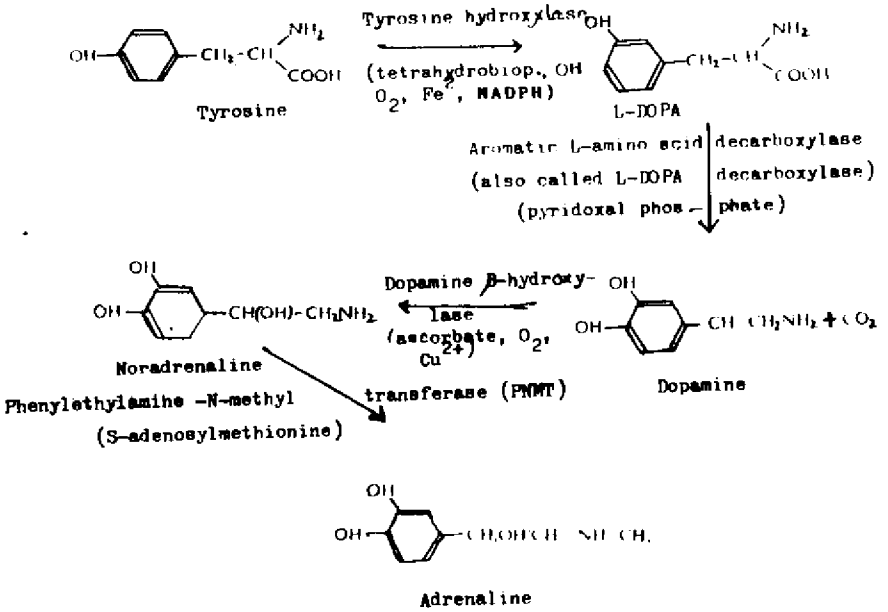

Fig.1

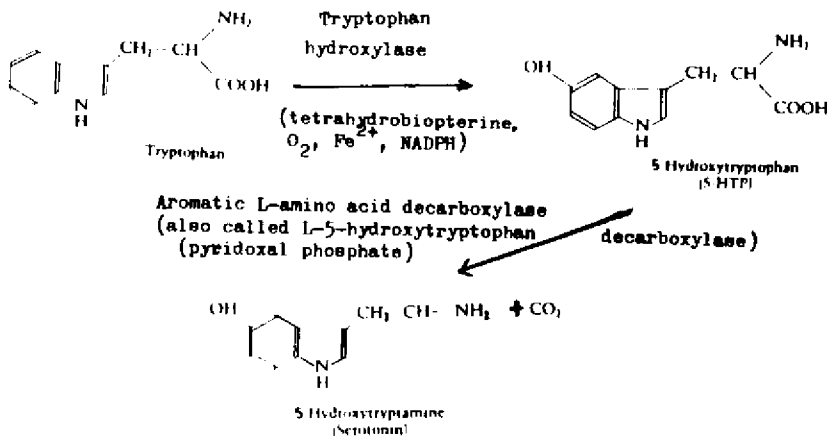

Fig.2

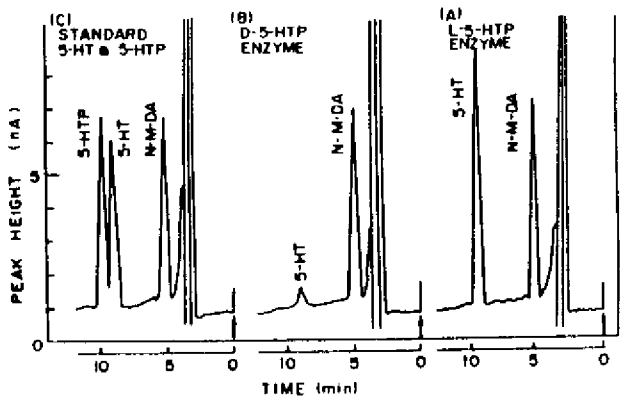

Fig.3

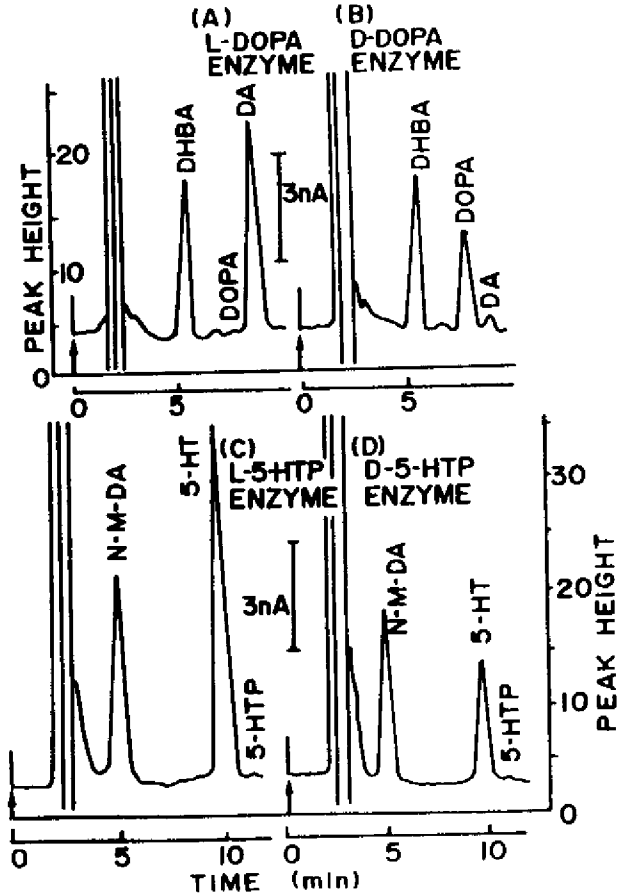
(A)
L-DOPA
ENZYME
(B)
D-DOPA
ENZYME
DHBA
DA
DOPA
3nA
DHBA
DOPA
DA
PEAK HEIGHT
20
10
0
0
5
0
5
N-M-DA
5-HT
(C)
L-5-HTP
ENZYME
(D)
D-5-HTP
ENZYME
3nA
N-M-DA
5-HT
5-HTP
5-HTP
30
20
10
0
PEAK HEIGHT
0
5
10
0
5
10
TIME (min)

Fig. 4

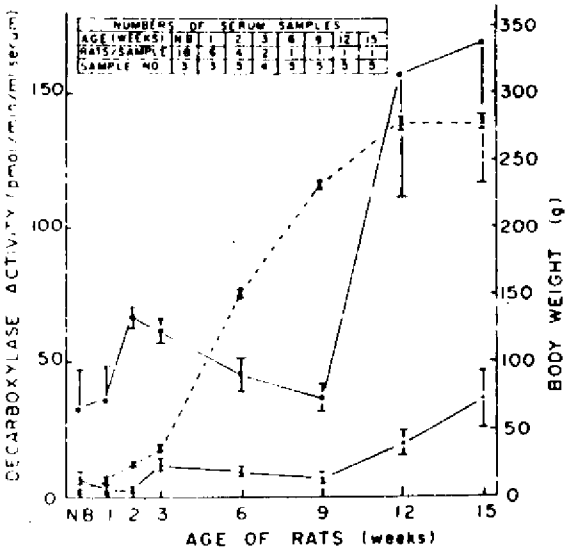
NUMBERS OF SERUM SAMPLES
AGE (WEEKS) NB 1 2 3 6 9 12 15
RATS/SAMPLE 16 6 4 2 1 1 1 1
SAMPLE NO 3 3 5 4 5 5 5 5
DECARBOXYLASE ACTIVITY (pmol/min/ml serum)
150
100
50
0
350
300
250
200
150
100
50
0
BODY WEIGHT (g)
NB 1 2 3 6 9 12 15
AGE OF RATS (weeks)

Fig. 5

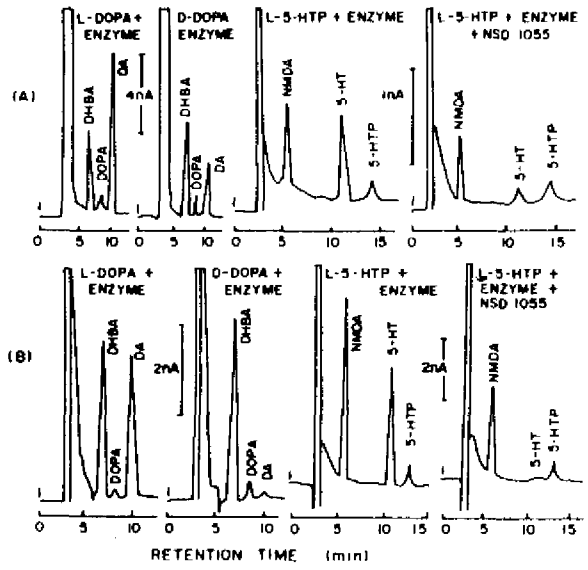

Fig. 6

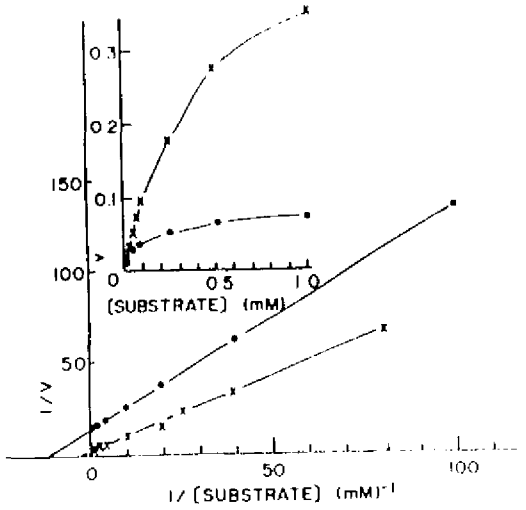

Fig. 7

a b c d e f g

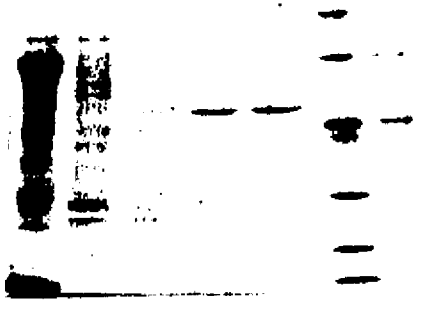

Fig. 8

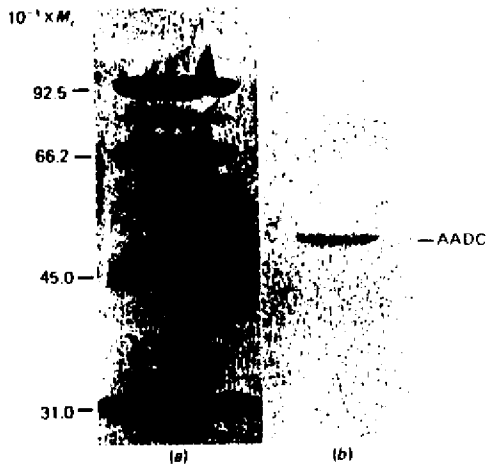
$10^{-3} \times M_r$
92.5
66.2
45.0
31.0
AADC
(a)
(b)

Fig. 9

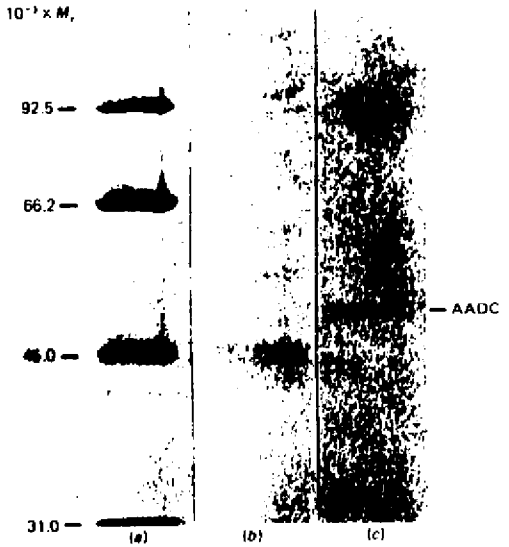
$10^{-3} \times M_r$
92.5
66.2
45.0
31.0
AADC
(a)
(b)
(c)

Fig. 10