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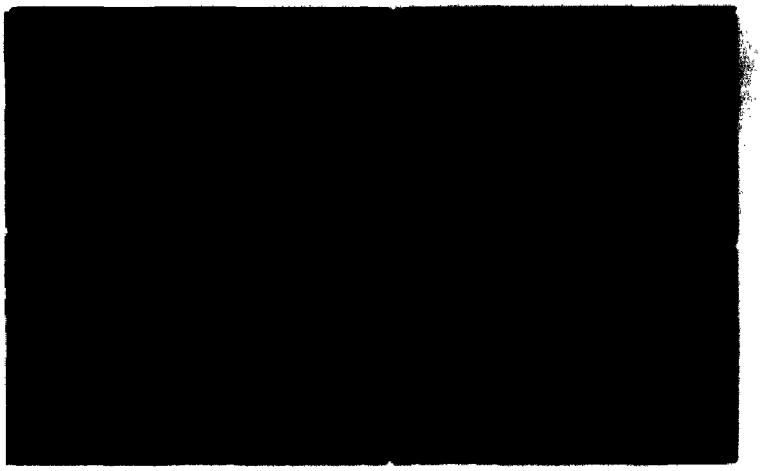

TNO

BINDING OF RADIATION-INDUCED PHENYLALANINE RADICALS TO DNA: INFLUENCE ON THE BIOLOGICAL ACTIVITY OF THE DNA AND ON ITS SENSITIVITY TO THE INDUCTION OF BREAKS BY γ-RAYS

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Werkgebied 2 : Bescherming tegen (ioniserende) straling en chemische agentia

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Binding of radiation-induced phenylalanine radicals to DNA: Influence on the biological activity of the DNA and on its sensitivity to the induction of breaks by γ-rays

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Motivering en toelichting

Bij bestraling van een levende cel met ioniserende straling (bijv. röntgenstraling) treden er in de cel zeer veel verschillende reacties op die leiden tot mutaties en, in het ergste geval, tot de dood van de cel.

Aangenomen wordt dat het DNA, de erfelijke substantie van de cel, het meest gevoelige doelwit is voor dergelijke straling. De stralingsschade in DNA kan door directe wisselwerking van de straling met DNA worden teweeggebracht of door de zeer reactieve radikalen die door de straling in de directe omgeving van het DNA worden gevormd. Enkele jaren geleden is in dit laboratorium reeds aangetoond dat door straling gevormde radikalen van fenylalanine, een aminozuur dat normaliter in de cel voorkomt, in staat zijn zowel enkelstrengig als dubbelstrengig DNA te inactiveren.

In het onderhavige rapport wordt aangetoond dat vele fenylalanine-radikalen aan het DNA worden gebonden zonder het DNA te inactiveren. Dit geldt zelfs voor enkelstrengig DNA, hetgeen merkwaardig is daar algemeen wordt aangenomen dat herstel van stralingsschade in de cel alleen kan plaatsvinden als het DNA zich in de dubbelstrengige vorm

bevindt. Ook kunnen fenylalanine-radikalen breuken in de strengen van het DNA veroorzaken.

Fenylalanine dat door straling aan DNA is gebonden, bleek het DNA gedeeltelijk te beschermen tegen de vorming van breuken in de strengen van het DNA door straling. Ook produkten die door bestraling uit fenylalanine ontstaan, bleken een extra beschermend effect te hebben tegen de vorming van breuken.

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Summary

When an aqueous solution of double-stranded DNA of bacteriophage PM2 containing phenylalanine and saturated with N_2O is irradiated with γ-rays, radiation-induced phenylalanine radicals are bound covalently. Under the conditions used about 25 phenylalanine molecules may be bound per lethal hit. Also for single-stranded PM2 DNA most of the phenylalanine radicals bound are non-lethal. Evidence is presented that in double-stranded DNA an appreciable fraction of the single-strand breaks is induced by phenylalanine radicals. Radiation products of phenylalanine and the phenylalanine bound to the DNA decrease the sensitivity of the DNA to the induction of single-strand breaks. There are indications that the high efficiency of protection by radiation products of phenylalanine is due to their positive charge, which will result in a relatively high concentration of these compounds in the vicinity of the negatively charged DNA molecules.

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1. Introduction

Ionizing radiation may induce damage in the DNA of a living cell by direct interaction with the DNA or by inducing deleterious radicals in its vicinity. These radicals may be both free water radicals and radicals of other cell constituents. De Jong, Loman and Blok (1972 a and b) have shown that in solution the single-stranded DNA of bacteriophage ØX174 and the double-stranded replicative form (RF) DNA of this phage are inactivated by radiation-induced phenylalanine (Phe) radicals. In the present paper evidence is presented that many Phe radicals may form a covalent bond with the DNA without inactivating it, even in the case of single-stranded DNA. The Phe bound was found to have a protective effect against induction of single-strand breaks.

2. Materials and Methods

2.1. *DNA*

Unlabelled and ^{3}H-labelled DNA of bacteriophage PM2 have been prepared and its biological activity determined as described previously (Van der Schans, Weyermans and Bleichrodt, 1971; Van der Schans, Bleichrodt and Blok, 1973). The DNA was purified by gelfiltration on Sephadex G-25 (coarse) using as eluent 10^{-2} M phosphate buffer (pH 7.4, prepared with triply distilled water). RF-DNA of bacteriophage ØX174 was prepared according

to Godson and Vapnek (1973) but instead of alcohol precipitation concentration of the DNA solution was achieved by vacuum evaporation. The biological activity was assayed according to the method of Guthrie and Sinsheimer (1963) for single-stranded ØX174 DNA.

2.2. *Chemicals*

Phe (L-phenylalanine, A grade) was obtained from Calbiochem, 3,4-dihydroxy-phenylalanine (dopa), dopamine-HCl and tyrosine from Fluka A.G., tyramine-HCl and β-phenylethyl amine-HCl from Hoffmann, La Roche and Co, and radioactively labelled Phe from the Radiochemical Centre, Amersham. To remove the alcohol in the samples of ^{3}H-labelled Phe (L-phenyl 2,3-^{3}H-alanine, 20 Ci/mmol, chromatographic purity according to the manufacturer, 99 - 100 per cent) and ^{14}C-labelled Phe (uniformly labelled, 552 mCi/mmol, chromatographic purity as reported by the manufacturer 98 per cent and according to our own measurement ⩾ 98 per cent), they were freeze-dried and redissolved in triply distilled water.

2.3. *Irradiation*

Irradiation of solutions of DNA (about 15 µg/ml) in 10^{-2} M phosphate buffer containing Phe (pH 6.0 or 7.2), was carried out at 0 °C with a ^{60}Co source (Gammacell 200, Atomic Energy of Canada Ltd). The dose rate (about 19 krad/min) was measured by $FeSO_4$ dosimetry. To convert radiation-induced hydrated electrons to OH radicals N_2O which was freed from traces of O_2 with a BTS catalyst (Badische Anilin und Soda Fabrik A.G., Ludwigshafen/Rhein), was led through the solution. The gas leaving the solution contained less than 0.002 per cent O_2, as measured with a Hersch cell (Hersch, 1960). After irradiation free radioactive Phe was removed by dialysis against 10^{-2} M phosphate buffer (pH 7.4) followed by gelfiltration on Sephadex G-25

or G-100, using the same buffer as eluent. For removal of unlabelled Phe the dialysis step was omitted.

2.4. *Sedimentation in sucrose gradients*

Sedimentations were carried out in sucrose gradients (5 - 23 per cent sucrose w/v in 1 M NaCl + 0.02 M Na_3-citrate) in polyallomer tubes (Van der Schans, Aten and Blok, 1969) at 5 °C, using an SW 27 rotor of a Spinco preparative ultracentrifuge or an SB 110 rotor of an International Equipment Company ultracentrifuge. After centrifugation gradients were fractionated to measure the distribution of the radioactivity (Van der Schans *et al.* 1973). The average numbers of single- and double-strand breaks per DNA molecule were calculated from the sedimentation profiles. To this end the formula of Van der Schans *et al.* (1969) for the sedimentation of linear molecules was modified.

For the production of a piece of linear DNA from a circular molecule two breaks are needed. If p breaks are induced at random in the circular molecule the probability P(m)dm that a linear molecule with a molecular weight between m and m + dm is produced, is given by

$$P(m)dm = (pdm/M).\ \exp(-pm/M)\int_0^M pd\mu/M$$

The ultraviolet light absorption or radioactivity E of the DNA as a function of the sedimentation coefficient s, assuming $s = km^{\alpha}$, satisfies the equation

$$CE(s) = mP(s) = mP(m)\frac{dm}{ds} = m^{2-\alpha}p^2.(\alpha kM)^{-1}.\exp(-pm/M)$$

where C is a normalization factor and α and k are constants. For the maximum in the E(s) profile in the sucrose gradient

$$p = (2-\alpha)M/m,$$

where m is the molecular weight corresponding to the maximum in the distribution function E(s).

The method has been calibrated with denatured T4 DNA, T7 DNA, untwisted PM2 DNA (i.e. molecules containing a single-strand break) and untwisted ØX174 RF-DNA, assuming that the molecular weights of these denatured DNA's are 65×10^6, 12.5×10^6, 3×10^6 and 1.7×10^6 respectively. The best values of the constants in $S^{sucr}_{20,w} = kM^{\alpha}$ were found to be $k = 0.013$ and $\alpha = 0.54$.

To assess the biological activity of single-stranded PM2 DNA rings, the DNA was centrifuged through alkaline glycerol gradients (5 - 25 per cent glycerol w/v in 1 M NaCl + 0.02 M Na_3-citrate, pH 12.1; Van der Schans *et al.* 1971). Fractions of the gradient of 15 drops (0.5 ml) were diluted 40 times with 10^{-2} M phosphate buffer and assayed for biological activity.

2.5. *Denaturation*

DNA was denatured by adding at 0 °C 0.06 ml 0.96 M NaOH to 0.50 ml of the DNA solution (final pH about 12.7). After 10 min at 0 °C the solution was neutralized slowly with 0.3 M HCl dissolved in neutral 0.2 M tris buffer. With this mild alkaline treatment breakage of radiation-induced alkali-labile bonds is prevented. This was shown by subjecting twisted covalently closed PM2 DNA which had been irradiated to introduce alkali-labile bonds to the alkaline treatment and measuring the average number of single-strand breaks before and after this treatment from the conversion of the covalently closed form into molecules containing one or more breaks.

2.6. *Chromatographic analysis of radiation products of phenylalanine*

15 mM Phe in 10^{-2} M phosphate buffer was irradiated with 600 or 1200 krad under N_2O and analysed with an LKB amino acid analyser No. 3201, using a pH gradient of 3.12 - 6.45. G(-Phe) was found to be 3.0. The G-value for the production of tyrosine was 0.28.

15 mM Phe + ^{14}C-Phe in 10^{-2} M phosphate buffer was irradiated with 1200 krad under N_2O. Samples were chromatographed in two dimensions on Whatman No. 4 paper for periods up to 16 hours, using n-butanol-acetic acid-water (4:1:5) and phenol saturated water, respectively. Reference compounds were dopa, dopamine, tyrosine, tyramine and β-phenylethyl amine. The spots were localized by ninhydrine spraying, the paper was subsequently cut into pieces which were put into 10 ml of toluene, containing 10^{-2} g/l POPOP and 6 g/l PPO, in low back-ground polyethylene vials, to measure the radioactivity with a Nuclear Chicago Mark II liquid scintillation counter (counting efficiency about 30 per cent).

3. Results

3.1. *Radiation-induced covalent binding of Phe to DNA*

Evidence that Phe is bound covalently to DNA under the influence of γ-irradiation is shown in figure 1. In sedimentation patterns of PM2 DNA irradiated in the presence of Phe, the radioactivity pattern follows the ultraviolet light absorption profile of the DNA in the gradient (Fig. 1b). In sedimentation patterns of unirradiated but otherwise similarly treated PM2 DNA, no radioactivity is found in the DNA peaks (Fig. 1a). If the irradiated PM2 DNA sample is denatured with alkali and sedimented through an alkaline sucrose gradient (Fig. 1c; the peak in this profile is broad because the broken single-strand pieces are polydisperse) again radioactivity is associated with the ultraviolet absorbing material. Further,

heating of the purified Phe-DNA complex before centrifugation up to 60 °C for 5 min did not show any decrease of the specific radioactivity of the Phe-DNA complex.

The amount of Phe bound did not differ measurably for concentrations of Phe between 5 and 15 mM during irradiation, it was proportional to dose and it amounted to about 80 Phe molecules per PM2 DNA molecule per 100 krad. With ^{14}C-labelled Phe the same amount of binding was observed. Also, for the circular RF-DNA of bacteriophage ØX174 the amount of binding of Phe per unit of molecular weight was found not to differ significantly from that of PM2 DNA.

Because of the high radiation dose applied the question may be asked whether the radioactive material bound to the DNA originates from radicals induced by the γ-rays in degradation products of Phe instead of in Phe itself. The linear relationship between the amount of radioactivity associated with DNA and radiation dose, and the fact that the amount of Phe destroyed at 600 krad is only 15 per cent, does not favour an affirmative answer.

In solution equilibria exist between negatively charged (Phe^-), uncharged (Phe and $Phe^\pm$) and positively charged Phe molecules (Phe^+). Between pH 6 and 7.2 more than 99 per cent of the Phe molecules is uncharged, so the fraction of uncharged Phe molecules does not vary much by lowering the pH to 6. The concentration of the charged Phe molecules, however, will increase strongly. The amount of Phe bound by irradiation at pH 6 was found to be about twice that after irradiation at pH 7.2. In this pH range the reaction rates of Phe with water radicals do not strongly depend on pH (Anbar and Neta 1967; Lichtin and Shafferman 1974), so that the ratio of the numbers of Phe radicals and water radicals induced initially that can react subsequently with DNA will not differ at pH 7.2 and 6.0.

3.2. *Dependence of the induction of single-strand breaks and of the inactivation of PM2 DNA on Phe concentration*

In figure 2a the dose needed to induce on the average one single-strand break per PM2 DNA molecule ($D_{37,ss}$; measured at low doses) has been plotted against the concentration of Phe. At pH 7.2 $D_{37,ss}$ increases linearly with the concentration of Phe initially, but the curve becomes less steep above 35 mM. This is an indication that Phe radicals can induce single-strand breaks (Cf. de Jong et al. 1972 a, for a similar argument with regard to the lethality of Phe radicals for ØX174 DNA).

At pH 6.0 $D_{37,ss}$ is nearly independent of the concentration of Phe above 15 mM Phe and much lower than at pH 7.2. Since in the absence of Phe a pH change from 7.2 to 6.0 does not influence the yield of single-strand breaks significantly, this suggests that Phe radicals contribute substantially to the induction of single-strand breaks.

In a DNA solution containing 15 mM Phe and of pH 6.0 an increase of the ionic strength to 1 M NaCl resulted in a decrease of the induction of single-strand breaks by a factor of about 2. At pH 7.2 an increase of the ionic strength did not influence the efficiency of induction of single-strand breaks by γ-rays significantly.

Figure 2b shows the 37 per cent survival dose of PM2 DNA as a function of the concentration of Phe at pH 7.2. Contrary to the results of De Jong et al. (1972 a), obtained with the single-stranded DNA of the bacteriophage ØX174, the curve does not reach a horizontal plateau. This is an indication that there is some scavenging of lethal Phe radicals.

3.3. *Protective effect of radiation products of Phe and of bound Phe on the production of single-strand breaks*

In figure 3a the average number of single-strand breaks per PM2 DNA molecule has been plotted as a function of dose for pH 7.2. The curve shows an initial slope of 9.0 (Cf. fig. 3b) and a final slope of 2.2 single-strand breaks per molecule per 100 krad. Part of this decrease in slope is probably due to the fact that one or more radiation products of Phe protect the DNA more efficiently than Phe against induction of single-strand breaks. As shown in figure 3b, Phe which has been pre-irradiated with a dose of 600 krad gives an extra protection by a factor of 2.2. Since the ratio of the initial and final slopes of the curve in figure 3a is 4.1, this suggests that bound Phe also causes an extra protection and that by a factor of about 4.1/2.2. Evidence for the latter hypothesis is provided by experiments which showed that Phe bound to DNA protects against single-strand breaks induced by water radicals. In these experiments DNA was irradiated in the presence of Phe and the Phe-DNA complex induced was purified, mixed with a small amount of uncomplexed ^{3}H-labelled PM2 DNA and the mixture in pure phosphate buffer was irradiated again. After alkaline denaturation samples were subjected to centrifugation through neutral sucrose gradients (the number of breaks had to be determined with denatured DNA because no Phe-DNA complex in the twisted form was left after irradiation). From the ultraviolet light absorption pattern in the gradients the number of breaks induced in the Phe-DNA complex was calculated and from the radioactivity profile the number induced in the uncomplexed DNA. Figure 4 shows that the efficiency of induction of single-strand breaks in the Phe-DNA complex is about 25 per cent smaller than that in the uncomplexed DNA.

Similar results were obtained when a small amount of ^{3}H-labelled Phe-DNA complex was irradiated in the presence of a relatively large amount (sufficient to measure UV absorption) of untreated unlabelled PM2 DNA.

The radiation treatment necessary to produce the Phe-DNA complex induces also breaks. This is the reason that in figure 4 the intercept for the Phe-DNA complex is larger than that for the DNA that has not been pre-treated. In a control experiment the uncomplexed DNA was pre-irradiated in buffer with a dose that yielded approximately the same number of breaks as in the Phe-DNA complex. The results led to the same conclusion as that obtained from figure 4.

Whether the protection by bound Phe against single-strand breaks which are induced by Phe radicals is more efficient than that against single-strand breaks which are induced by water radicals (a factor of 4.1/2.2 has to be accounted for), is difficult to determine, because irradiation of a mixture of purified Phe-DNA complex and unirradiated ^{3}H-labelled DNA in the presence of Phe yields non-linear curves for the number of breaks against dose (Cf. fig. 3a). Furthermore, the dose needed to induce sufficient additional breaks in the Phe-DNA complex for a determination of the efficiency of break induction is so high in the presence of Phe that reactions of radiation products of Phe cannot be neglected.

In order to investigate what radiation products of Phe may confer an extra protection against the induction of single-strand breaks, the products of Phe irradiated under the conditions used in this paper had to be analysed first (see 2.6). Chromatographic analysis showed that besides small amounts of unidentified products, tyrosine, β-phenylethyl amine, tyramine and dopamine were produced by γ-rays. No dopa was detected (Cf. Wheeler and Montalvo 1969). At 1200 krad about 30 per cent of the Phe had been converted to products, the amount of tyrosine induced was about 2 per cent of the initial amount of Phe. The amount of the other products was 1 per cent or less at this dose.

In subsequent experiments small amounts of the above-mentioned compounds were added to solutions of PM2 DNA in 15 mM Phe. As shown in the Table, β-phenylethyl amine, tyramine and dopamine, of which most molecules possess a positive charge in solution, protected significantly against γ-rays, whereas tyrosine and dopa, which are mostly uncharged, gave much less or no protection. The protective effect of small amounts of the positively charged molecules could be suppressed by increasing the ionic strength of the solution (1 M NaCl). This suggests that these molecules protect at such low concentrations because their concentration in the vicinity of the negatively charged DNA is relatively high. The efficient protection of DNA by diamino-disulphides has been ascribed, at least partly, to a similar effect (Jellum 1966).

3.4. *The influence of bound Phe on the biological activity of PM2 DNA*

As pointed out in section 3.1, the amount of bound Phe was found to be constant for concentrations of Phe during irradiation between 5 and 15 mM and to amount to 80 Phe molecules per DNA molecule per 100 krad. In the same concentration range the 37 per cent survival dose varied from 18 to 33 krad. This means that 14 to 26 Phe molecules were bound per lethal hit.

Since this efficiency of inactivation is rather small, the influence of binding of Phe radicals on the biological activity of single-stranded PM2 DNA was also determined. Double-stranded PM2 DNA was irradiated with various doses in the presence of 15 mM Phe, denatured with alkali, sedimented through alkaline glycerol gradients and the gradient fractions containing the single-stranded PM2 DNA rings were tested for biological activity. From the biological activity of the single-stranded rings as a function of dose the 37 per cent survival dose was found to be 13 krad. This corresponds to about 5 molecules of Phe bound per lethal hit that results from damages other than single-strand breaks (single-stranded PM2 DNA containing a break is not biologically active; Van der Schans and Bleichrodt 1974).

4. Discussion

Byfield, Lee and Bennet (1970) presented evidence that irradiation of a mixture of calf thymus DNA (about 1 mg/ml) and radioactively labelled leucine (5×10^{-4} mg/ml) under mainly anoxic conditions causes covalent binding of the amino acid to DNA. The amount of amino acid bound was about 10^4 times smaller, however, than that for Phe as presented in this paper. Byfield *et al.* (1970) found for other amino acids a similar radiation-induced binding (for Phe only binding to chromatin has been reported).

Per lethal hit 14 to 26 Phe molecules are bound to double-stranded PM2 DNA. This means that more than 92 per cent of the Phe bound is non-lethal. In similar experiments with RF-DNA of phage ØX174 5 to 10 Phe molecules were found to be bound per lethal hit. The differences between the two DNA's may be due to differences in the capacity of the corresponding hosts (*Escherichia coli* K12 for RF-DNA and *Pseudomonas* BAL 31 for PM2 DNA) to repair radiation damage. That some Phe radicals do inactivate DNA has already been shown by De Jong *et al.* (1972 a and b) for single- and double-stranded ØX174 DNA.

In single-stranded PM2 DNA at least 80 per cent of the Phe bound is non-lethal. Either this type of damage in single-stranded DNA can be repaired or it does not impair progeny formation. In this connection it is relevant to mention that Swinehart and Cerutti (1975) obtained evidence that the number of damaged thymine bases in the single-stranded DNA of γ-irradiated bacteriophage ØX174 exceeded the number of lethal hits by two orders of magnitude. Lafleur, Loman and Blok (1975) concluded from the relatively high D_{37} for inactivation of thouroughly purified ØX174 DNA in solution that many of the primary lesions in the DNA do not lead to inactivation.

The dose needed to induce on the average one single-strand break per molecule ($D_{37,ss}$) does not increase linearly with the concentration of Phe (fig. 2a). Moreover the yield of single-strand breaks is strongly increased when the pH is lowered from 7.2 to 6, a phenomenon which is not observed in the absence of Phe. These observations suggest that besides water radicals secondary Phe radicals play a role in inducing single-strand breaks.

It may be asked which part of the single-strand breaks is due to water radicals and which one to Phe radicals. As estimated in the appendix at pH 7.2 in the presence of 15 mM Phe the fraction of single-strand breaks induced by Phe radicals amounts to about 26 per cent, at pH 6.0 it is of the order of 91 per cent. The fact that addition of salt had a much stronger effect on the induction of single-strand breaks at pH 6.0 than at pH 7.2, suggests that at pH 6.0 mainly positively charged Phe radicals are involved in the induction of single-strand breaks.

The protection against the induction of single-strand breaks by radiation products of Phe (after a radiation dose of 600 krad) at pH 7.2 amounts to a factor of 2.2 (fig. 3b). The ratio of the initial and final slopes of the curve for induced breaks against dose in figure 3a is 4.1. This suggests that a protection by a factor of about 1,9 has to be ascribed to bound Phe. However, as shown in figure 4, bound Phe protects only by a factor of 1.3 against single-strand breaks induced by free water radicals. This suggests that bound Phe protects more efficiently against the breaks induced by Phe radicals.

When a is the fraction of breaks induced by Phe radicals and $(1-a)$ that induced by water radicals, then $(1-a)/1.3+a/x = 1/1.9$, where x is the protection factor for bound Phe against breaks induced by Phe radicals. According to this equation at least 32 per cent of the single-strand breaks is induced by Phe radicals. The reason for the discrepancy between this

value and that of 26 per cent mentioned above is not known at present.

It is remarkable that a relatively small amount of bound Phe seems to protect so strongly against the production of single-strand breaks. The strong protection by 0.2 mM of the positively charged radiation products of Phe in a solution containing 15 mM Phe (see Table) is also surprising. It is easily calculated that at the ionic strength used in the experiments of the Table the amount of the added compounds bound electrostatically by the DNA, is at most 300 molecules per PM2 DNA molecule. The DNA which contains about 20,000 nucleotides is protected, however, by a factor of 2.5.

The induction of double-strand breaks has not been investigated. Because the production of double-strand breaks is about two orders of magnitude smaller than that of single-strand breaks, rather high radiation doses are needed to detect any double-strand breaks. At these high doses radiation products of Phe cannot be neglected.

Summarizing, it may be concluded from the data presented that :

a) Phenylalanine radicals, formed in the absence of oxygen, bind covalently to PM2 DNA; more than 90 per cent of this type of damage is non-lethal.

b) Evidence is obtained that under the irradiation conditions used an appreciable fraction of the single-strand breaks results from reaction of Phe radicals with DNA.

c) Radiation products of Phe give an increased protection against breaks. The efficiency of protection of these products is probably due to their positive charge.

d) Bound Phe reduces the yield of radiation-induced single-strand breaks. This and the preceding observation result in a non-linear induction of single-strand breaks with dose.

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APPENDIX

The experiments were performed under N_2O, which converts e^-_{aq} into $OH^\cdot$. If the contribution of $H^\cdot$ to the induction of breaks is neglected as a first approximation, the yield of breaks per 100 eV G(ss) is the sum of the yields of breaks due to $OH^\cdot$ and $Phe^\cdot$ radicals.

$$G(ss) = G(ss_{OH^\cdot}) + G(ss_{Phe^\cdot})$$

The first term satisfies.

$$G(ss_{OH^\cdot}) = G(OH^\cdot) \frac{k_1[DNA]}{k_1[DNA] + k_2[Phe]} \cdot \phi_{OH^\cdot} ,$$

where $G(OH^\cdot)$ is the primary yield of $OH^\cdot$, $\phi_{OH^\cdot}$ the fraction of the $OH^\cdot$ radicals reacting with DNA that induce a break, and k_1 and k_2 the reaction rate constants of the reactions of $OH^\cdot$ with DNA and Phe respectively. The first term in the denominator is small compared to the second at not too low concentrations of Phe, i.e.

$$G(ss_{OH^\cdot}) = \frac{A}{[Phe]} ,$$

where A is a constant.

If $Phe^\cdot$ radicals are not scavenged by Phe molecules

$$G(ss_{Phe^\cdot}) = G(OH^\cdot) \frac{k_2[Phe]}{k_1[DNA] + k_2[Phe]} \phi_{Phe^\cdot} ,$$

where $\phi_{Phe^\cdot}$ is the fraction of $Phe^\cdot$ radicals inducing breaks. At not too low concentrations of Phe this can be simplified to

$$G(ss_{Phe^\cdot}) = B = \text{constant}$$

Since the reciprocal of $D_{37,ss}$ is proportional to G(ss),

$$1/D_{37,ss} = \frac{A'}{[Phe]} + B' ,$$

where A' and B' are constants.

The curve in figure 2b does not show a horizontal plateau, indicating that some scavenging of Phe$^{\cdot}$ by Phe occurs at pH 7.2. From the slope of the linear part of the curve it follows that the fraction of Phe$^{\cdot}$ not scavenged by Phe is about $97/(97 + [Phe])$. If it is assumed that the same holds for Phe$^{\cdot}$ radicals inducing single-strand breaks, the latter formula becomes

$$1/D_{37,ss} = \frac{A'}{[Phe]} + B'\frac{97}{97 + [Phe]}$$

This formula can also be written as

$$\frac{[Phe]\,(97 + [Phe])}{D_{37,ss}} = 97A' + (A' + 97B')[Phe] .$$

When the left hand part of this equation is plotted against Phe, using the data of figure 2a, for both pH values straight lines are obtained (Fig. 5). From the curve for pH 7.2 A' and B' can be obtained, which in turn allow calculation of $G(ss_{Phe^{\cdot}})$ and $G(ss_{OH^{\cdot}})$. At this pH and a concentration of Phe of 15 mM the fraction of single-strand breaks induced by Phe$^{\cdot}$ radicals appears to be 0.26.

The fraction of Phe$^{\cdot}$ not scavenged by Phe, as estimated from the final slope of the curve for pH 6.0 in figure 2a, is of the same order as that calculated from the curve in figure 2b. Assuming that the above

formula holds also for pH 6.0 (Cf. fig. 5), the fraction of single-strand breaks induced by Phe• radicals in a solution containing 15 mM Phe of pH 6.0 appears to amount to 0.91.

added compound	single-strand breaks induced (relative amounts)*
-	1
tyrosine	0.83
dopa	0.88
tyramine	0.41
dopamine	0.35
β-phenylethyl amine	0.35
β-phenylethyl amine + 1 M NaCl	0.94
1 M NaCl	0.96

* averages of two or more independent experiments. γ-ray doses applied depended on the efficiency of induction of breaks and were 0, 15 and 30 krad or 0, 30 and 60 krad.

Table. Influence of small amounts of different compounds on the induction of single-strand breaks in PM2 DNA (10 µg/ml), irradiated by γ-rays in the presence of 15 mM phenylalanine. The concentration of the added compounds was 0.2 mM

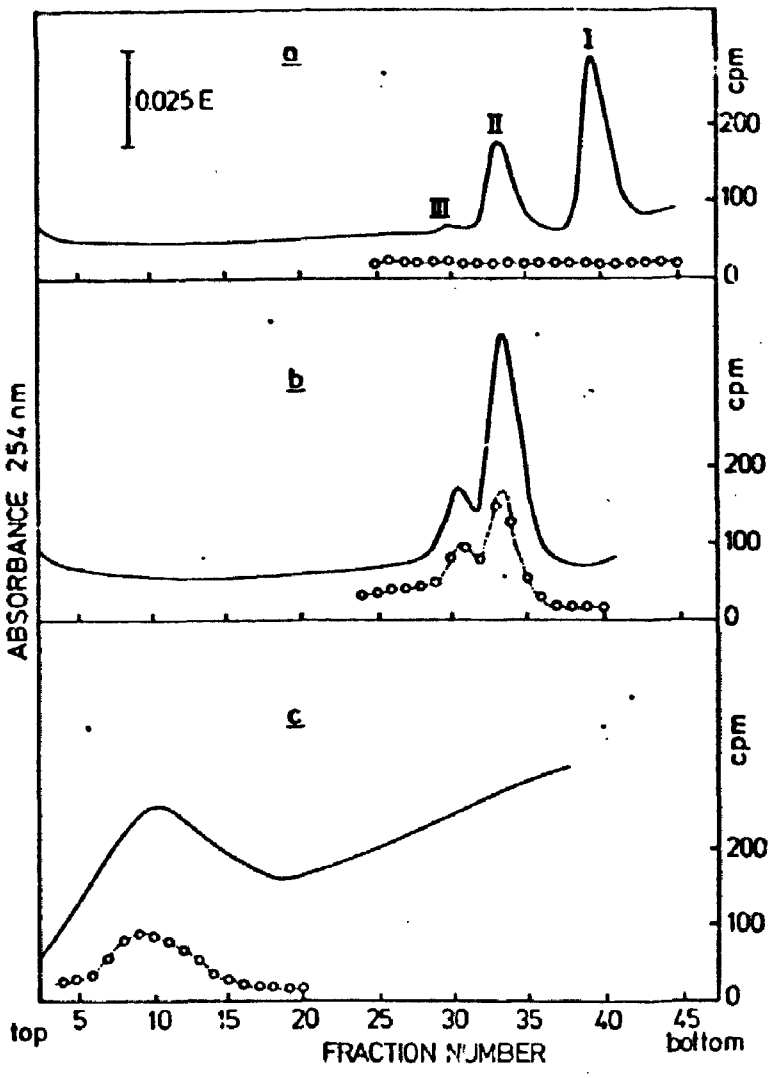

Figure 1. Sedimentation patterns of PM2 DNA, irradiated in the presence of ^{3}H-labelled Phe. The DNA (15 µg/ml) was irradiated in 10^{-2} M phosphate buffer containing 15 mM Phe + 60 µCi/ml ^{3}H-Phe and purified by dialysis and gelfiltration before centrifugation. The position of the different components is indicated by the corresponding Roman numerals; I = circular, twisted PM2 DNA; II = PM2 DNA containing one or more single-strand breaks; III = PM2 DNA containing a double-strand break. a : unirradiated, neutral gradient; b : irradiated with 600 krad, neutral gradient; c : irradiated with 600 krad, alkaline gradient, pH 12.1. The solid curves represent the absorption patterns, the dotted curves the radioactivity measurements (not corrected for back-ground of about 15 cpm). Alkaline gradients always show a relatively steep back-ground absorbance compared with neutral gradients.

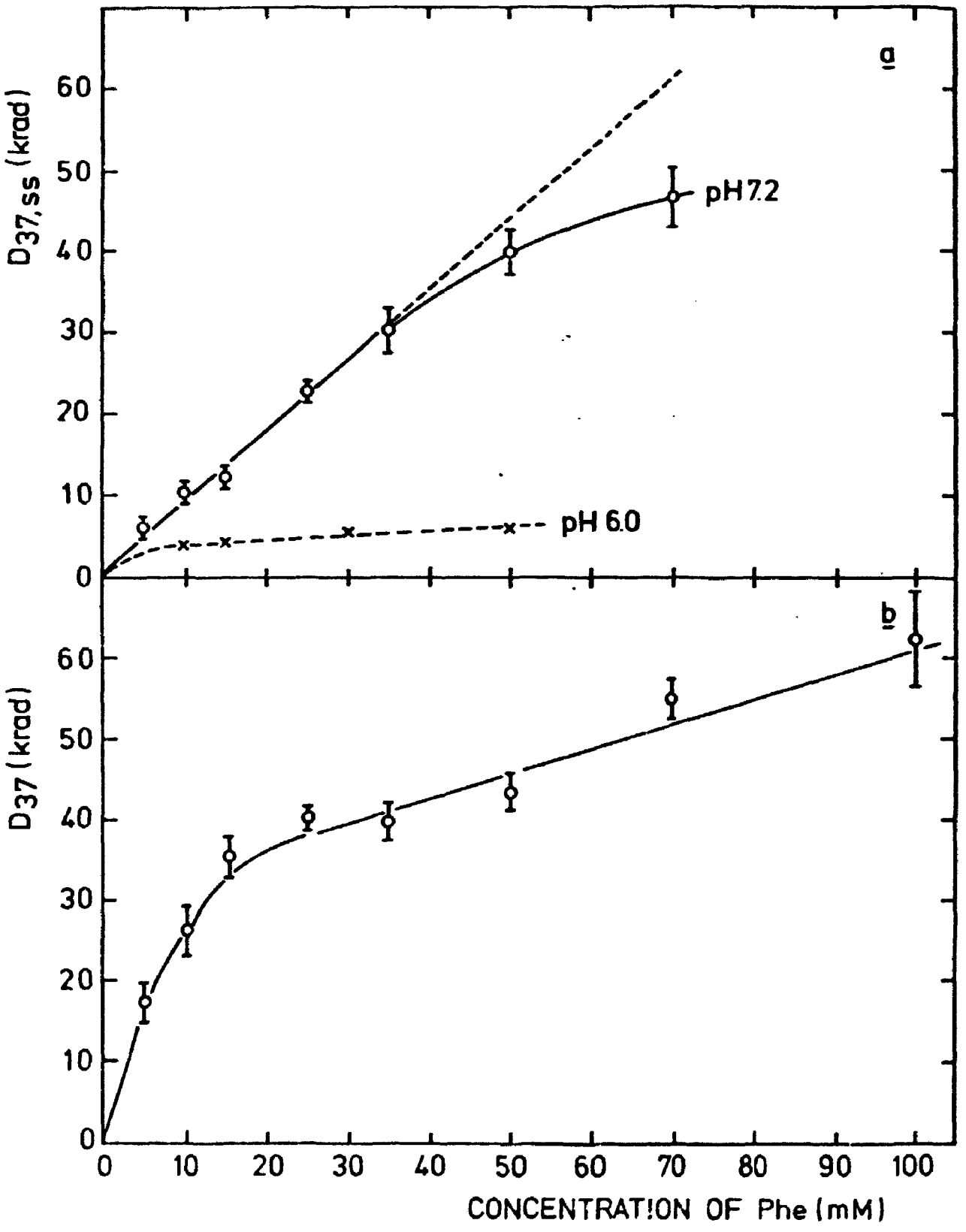

Figure 2. a: The dose needed to induce on the average one single-strand break per PM2 DNA molecule ($D_{37,ss}$) versus the concentration of Phe. DNA concentration 15 µg/ml; O—O, pH 7.2; X- -X, pH 6.0.

b: 37 per cent survival dose (D_{37}) for the biological activity of double-stranded PM2 DNA versus the concentration of Phe. DNA concentration 15 µg/ml; pH 7.2.

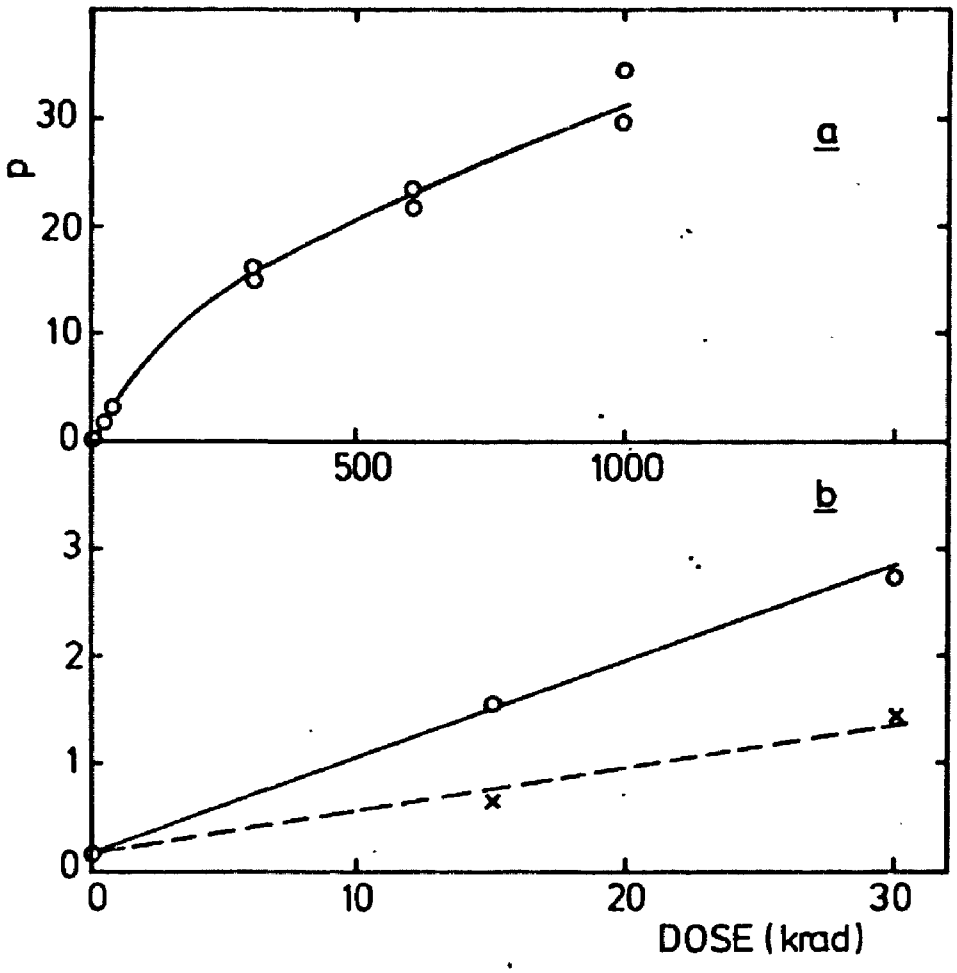

Figure 3. The average number of single-strand breaks per PM2 DNA molecule (p) versus γ-ray dose. a) PM2 DNA in 10^{-2} M phosphate buffer containing 15 mM Phe. b) Solid curve: PM2 DNA in buffer containing 15 mM Phe; broken curve: PM2 DNA in buffer containing 15 mM Phe which was pre-irradiated with 600 krad. DNA concentration: 15 μg/ml, pH 7.2.

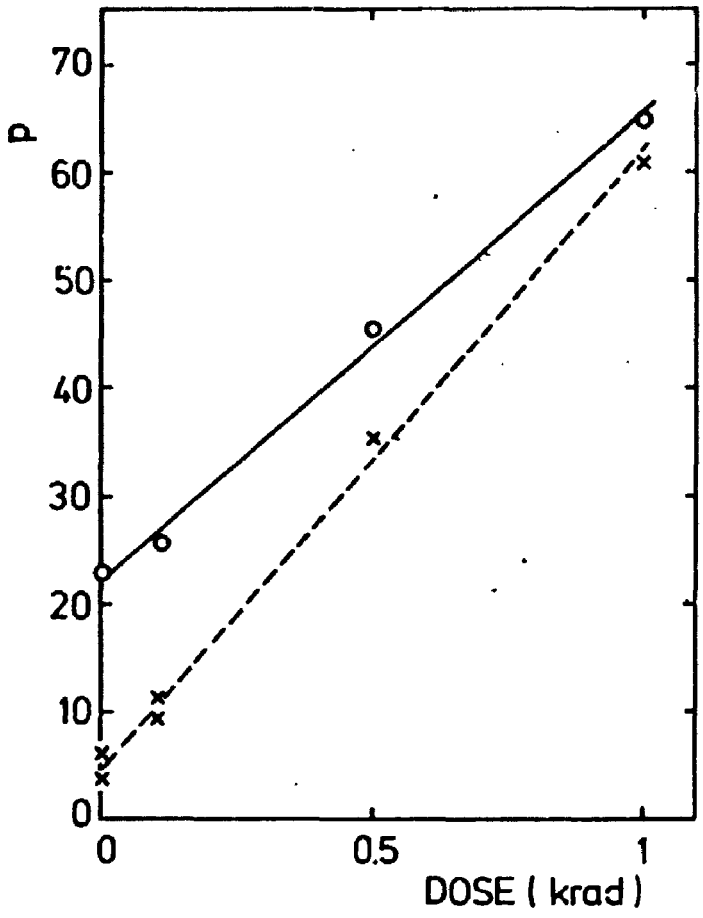

Figure 4. The average number of single-strand breaks per PM2 DNA molecule (p) versus γ-ray dose. A mixture of PM2 DNA containing bound Phe and ^{3}H-labelled PM2 DNA was irradiated with various doses and samples were denatured and subsequently centrifuged through neutral sucrose gradients. From the UV absorption profile of the Phe-DNA complex and the radioactivity pattern of the ^{3}H-labelled DNA the average number of single-strand breaks was calculated for these DNA's. The Phe-DNA complex was obtained by irradiating PM2 DNA in the presence of 15 mM Phe with 600 krad, it was purified by gelfiltration on Sephadex G-25 with 10^{-2} M phosphate buffer as eluent. Solid curve : Phe-PM2 DNA complex; broken curve : ^{3}H-labelled PM2 DNA.

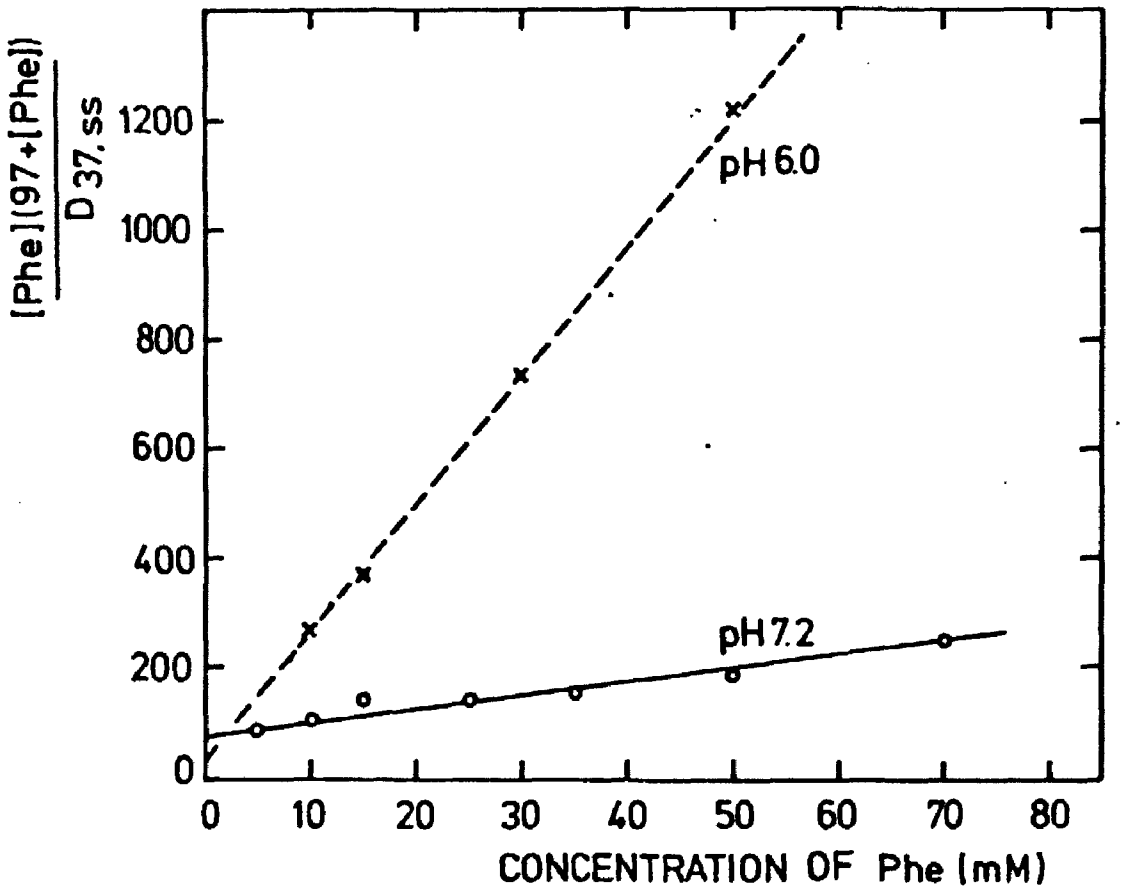

Figure 5. $\frac{[Phe](97 + [Phe])}{D_{37,ss}}$ as a function of the concentration of Phe

O——O pH 7.2; X- - -X pH 6.0. Same data as in figure 2a.

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