

**ANOMALOUS H / D ISOTOPE EFFECT IN HYDROGEN BONDED SYSTEMS
: H-BONDED CYCLIC STRUCTURES AND TRANSFERS OF PROTONS**

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The systematic H / D substitution is a precious tool to obtain information on the dynamics of H-bonds. It is particularly useful in IR spectroscopy where H-bonds are at the origin of particularly intense and specific bands and where the particularly great value for the m_D / m_H ratio ensures strongly marked effects. In most H-bonded systems the effects of these substitutions are normal, in the sense that they are at the origin of bands having intensities, centers (of intensity) and widths smaller in D-bonds by a factor close to $\sqrt{2}$ as compared to H-bonds. In some systems as carboxylic acid dimers, however, anomalous ratios of intensities are found upon such a substitution. Their origin is still obscure. Experimental results suggest that such anomalous ratios have much to do with the cyclic structure of these systems. It leads to stressing an important property of H-bonded cyclic structures which is that they seem necessary for having transfers of protons between molecules through H-bonds in a neutral aqueous medium ($p_H=7$) at room temperature. The mechanism of such transfers of protons is still poorly known, but these transfers are now suspected to play a fundamental role in such widespread reactions as hydrolyses, peptide synthesis, etc...which may make them soon appear as being a crucial basic mechanism for reactivity of aqueous systems, particularly biological systems.

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The aim of this paper, which summarizes a conference on "Isotopic Effects" delivered at a Summer School organized by the University of Wroclaw is to present reflections on anomalous changes induced on intensities of IR bands upon substitution of the H-atom of H-bonds by D-atoms in vapours of carboxylic acids. Such changes have been measured some years ago and have not yet been satisfactorily explained. After a brief description I shall indicate a possible origin for this effect, which will lead to formulating some prospective views on transfers of protons through H-bonds.

The H / D substitution is particularly simple and easy to perform in the case of H-bonds. This is because we have then at our disposal a species, namely heavy water, which practically behaves as an infinite reservoir of D-bonds which easily and rapidly exchanges in species having A-H groups able to establish H(D)-bonds with D₂O. As a consequence of this easiness this H / D substitution is systematically used in the characterization and study of H-bonds.

Its effect is spectacular in IR spectroscopy, particularly for the bands connected with vibrations of H-atoms, where the doubling of the mass of the vibrating atom then fully appears. As an illustration of these effects we compare the IR spectra of normal and heavy water in Fig. 1 (the quantity represented is ϵ'' , the imaginary part of the dielectric constant), obtained using Attenuated Total Reflection (or ATR) techniques [1][2]. In the case of these particularly simple molecules (H₂O or D₂O), we have two main bands: one corresponding to stretching vibrations of H (D) atoms parallel to the O-H valence bond (O-H \cdots O) called ν_s ; one corresponding to vibrations of the H-O-H angle, called δ . The two ν_s bands extend around 3500 cm⁻¹ for ordinary water (around 2500 cm⁻¹ for heavy water) while δ extends around 1650 cm⁻¹ for ordinary water (1200 cm⁻¹ for heavy water). We may see that upon D / H substitution the integrated intensities, centers (of intensity) and widths (defined as the second centered moment) of these two bands vary in a ratio close to the square root of the effective masses of the two vibrators, that is close to $\sqrt{2}$ in this case. These are expected effects which generally appear in all spectra of H(D)-bonds. However, in H-bonds formed from more complicated molecules R-O-H \cdots O bending vibrations of the R-O-H angle δ almost always couple with other vibrations in the R radical (they no longer are normal modes), spreading this effect over many bands and making it difficult to observe for these δ bands. ν_s vibrations, on the contrary, remain normal modes (they vibrate independently of other vibrations) for almost every H-bonded system, thus giving most intense ν_s bands which are characteristic of H-bonds and display the same behaviour as in water upon deuteration.

ANOMALOUS ISOTOPE EFFECT

The above described effects are of a great interest to detect and characterize H-bonds.

This is their main informative interest. Anomalous, or unexpected effects, on the other hand, may convey more original and specific information on H-bonds. Among these anomalous effects one concerns integrated intensities of bands of H-bonded cyclic dimers such as found in vapours of carboxylic acids. Such dimers have a very simple structure which makes them appear as model systems of H-bonds (Fig.2). Measurements of integrated intensities of most of the IR bands of these species have shown that intensities of bands due to vibrations in the H-bonded cyclic dimers greatly exceeded intensities of corresponding bands in D-bonded cyclic dimers [3][4]. Ratios of intensities of ν_s bands in fully H-bonded and fully D-bonded dimers was for instance found equal to 1.9, which is to be compared to the value 1.25 for monomers having no H(D)-bonds and which is close to the expected value equal to the square root of the effective masses. Also ratios of $\vec{C} = \vec{\delta}$ stretching bands in fully H-bonded and fully D-bonded dimers was found equal to 1.3, whereas it has the expected value 1 in monomers. Other bands also displayed an anomalously high ratio of intensities between these two kinds of dimers, which was not found in corresponding monomers with no H-bonds.

Tentative explanations of these effects by anharmonic mechanisms, including both mechanical and electrical anharmonicities have failed to reproduce these effects, except in the case of ν_s bands, where a possible explanation may be found [5]. Electrical anharmonicity means that the electric dipole moment contains important terms of order higher than 1 in the coordinates of displacements of nuclei. Same anomalous effects have been measured for ν_s bands (intensities of other bands have not been measured) of adipic acid crystals [6] which contains similar H-bonded cyclic dimers. In opposition ratios of ν_s bands in crystals of CH_3COOH and CH_3COOD [7] have been found to be normal, that is to fall in the vicinity of $\sqrt{2}$. In these crystals, however, acetic acid molecules do not form H-bonded cyclic dimers but chains of monomers held by H-bonds. It consequently suggests that this anomalous ratio is due to the cyclic structures of these H-bonded dimers.

THE SPECIFICITY OF H-BONDED CYCLES

If true this latter assertion leads us to the following question: What is the specificity of these H-bonded cyclic dimers which might allow such anomalous effect to appear? Apparently they have no particularly special character. If one looks closer, one may see that they indeed have one: H-bonded cyclic dimers allow transfers of protons, or more precisely a certain kind of transfers of protons which occurs following the scheme represented in Fig.3a.

Before describing the various classes of transfers of protons and defining "resonance type" transfers which occur in H-bonded cycles, let us stress two points. The first one is that these transfers of protons really exist, as has been shown by Hayashi and coworkers [8][9] and

have been studied in considerable details[8]-[11]. The second point is that the mechanism by which such transfers may influence intensities of IR bands is not still precised. The main problem here is that it is not trivial to determine how such an essentially symmetric (with respect to the centre of inversion of the dimer) transfer of proton which does not induce any change in the dipole moment can influence intensities of IR bands which all correspond to antisymmetric vibrations. In other words the understanding of the influence of these transfers on IR intensities will have to go through determination of the mechanism by which these transfers break (may be momentarily) the symmetry of the dimer.

RESONANCE-TYPE TRANSFERS OF PROTONS

These cooperative transfers of protons in H-bonded cycles are of a special type which we call resonance-type to distinguish them from other types which may be more familiar and which are illustrated in Fig.3b. They occur between two resonant (having the same energy) ground electronic states of the dimer. The general problem of transferring protons through H-bonds [12] is that one should absolutely avoid creation of space charges as would appear in a simple transfer of the type $A-H \cdots B \rightarrow A^- \cdots H-B^+$. The appearance of such space charges is too costly in energy to give this reaction a reasonable probability to occur. It may be avoided by having anteriorly created these charges. This is what occurs in electrochemistry where, in an acidic or basic media such space charges as H_3O^+ or OH^- already exist and are all located on an hydrogen-bond network separated from counterions. A simple charge transfer, as displayed in Fig. 3b, reduces then to a migration of charges only, but not to creation of space charges. Transfers of the same kind occur in such biological processes as photochemistry or vision mechanism. Space charges are then indirectly created after capture of a visible photon by a pigment molecule[13][14][15], which allow storage of energy in the cell by accumulation of protons along a membrane. The way these protons migrate has been the object of many studies and are usually described as solitons [16]-[20].

Another way to avoid formation of space charges during a transfer of protons is to simultaneously perform a transfer of electrons. This is possible in H-bonded cyclic structures. In Fig.3a such a simultaneous transfer of electrons appears in the form of interchange of C=O and C-O bonds. These resonance transfers of protons may look as lacking generality, because they require a condition which may look quite severe and consequently able to appear in very special cases only: they occur within H-bonded cyclic structures. This is, however, a condition which is much less severe than it may at first sight look in the case of a widespread category of species which are aqueous media. In these media the presence of numerous H_2O molecules which are extremely avid of establishing H-bonds can easily fulfill this condition of closing H-bonded cycles.

These resonance transfers of proton have then an important asset: they do not require, as

other types of transfer do, to previously create space charges or excited electronic states which relax towards charged species. They are consequently able to occur in such media as neutral aqueous systems ($p_H=7$) at room temperature where other types of transfers cannot occur because of the absence of charged defects. We may then think that they are at the origin of the special reactivity of these neutral aqueous media at room temperature, particularly biological systems. In order to illustrate this point, let us take as an example such an ubiquitous reaction as the hydrolysis of an ester or a peptide group (or the reverse synthesis of proteins). For such reactions the rate-determining step is the formation of the so-called tetrahedral intermediate [21][22], at least in a neutral medium [23]. We shall here concentrate on this crucial step of the reaction, which is illustrated in Fig.4, neglecting subsequent steps.

In the case of an oversaturated neutral aqueous medium, that is when few H_2O molecules are available, this first step implies the formation of space charges. Taking as a criterion the no appearance of such space charges, this rate determining step has consequently a very low probability to occur (Fig.4a) and hydrolysis is hardly possible within such a simple scheme. Such a step becomes, however, possible in a basic medium (in an acidic medium as well, even if it proceeds with a different mechanism [24][25]), because it no longer implies creation of space charges, but only migration of these space charges, which is energetically much more favourable (Fig.4b). The result is that this step is then sufficiently rapid that it no longer constitutes the rate determining step [24].

Hydrolysis of an ester is nevertheless known to occur in a neutral medium. This is certainly so because appearance of space charges may be avoided by proton transfers inside an hydrogen-bonded cycle formed by one or more probably several water molecules (Fig.4c). It requires having an excess of water molecules which then act as kind of a catalyst for such reactions. The cost paid for such a mechanism is found in a supplement of entropy change (the mechanism has a more complicated pathway) which avoids passing over too high a barrier. In the case of amides, this step is apparently difficult to occur, certainly because of too severe steric conditions which may be due to too great a rigidity of C-N bonds [23]. It nevertheless occurs with the help of special enzymes, sterically fitted and at the same time able to transfer protons from one of their ends to another one, thus overcoming these steric hindrances by exactly adapting this transfer of proton inside H-bonded cycles to the geometry of peptide groups (Fig. 4d). Doing so, they perform the task of the "water wire" [26] of Fig.4c. Such an ability to transfer protons has been recognized to the triad group "serine-histidine-aspartic acid" of the active site of such enzymes as serine proteases [22][27].

CONCLUSION

Transfers of proton through H-bonds are a mechanism which are still poorly known but might reveal of a great fundamental importance. In particular resonance-type transfers which occur in H-bonded cycles may soon appear as crucial in the reactivity of aqueous systems, particularly biological systems. They give water a special role: that of establishing these H-

bonded cycles, which comes in addition to the well established role of water in biology at molecular level which is to act as an infinite reservoir of H-bonds allowing the appearance of specially flexible and adaptable molecular structures. This additional role of water in biology simply explains one of the most important H / D isotope effect which is that life, which occurs in ordinary water is hardly possible in a much similar species which has very close thermodynamical properties: heavy water. By simply slowing down that central mechanism of metabolic activity which is transfer of protons it leads to a considerable slowing down of the whole metabolic activity, thus making life impossible, except, may be, for such elementary organisms as bacteria.

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Captions to Figures

Fig. 1 - IR spectrum of ordinary (thick line) and heavy (thin line) waters at 40°C.

Fig. 2 - Monomers and dimers found in the vapour phase of carboxylic acids.

Fig.3 - Resonance type transfers of protons in H-bonded cyclic dimers (a) and diffusive type transfers of protons, as in an acidic medium (b).

Fig.4 - Hydrolysis of an ester, or amide: formation of a tetraedral intermediate (a); hydrolysis in a basic medium (b); hydrolysis in a neutral medium inside H-bonded cycles closed by water molecules(c); same mechanism for a peptide group in a neutral medium with the aid of an enzyme (schematically represented by dots) able to transfer a proton from one end of its active site to another one.

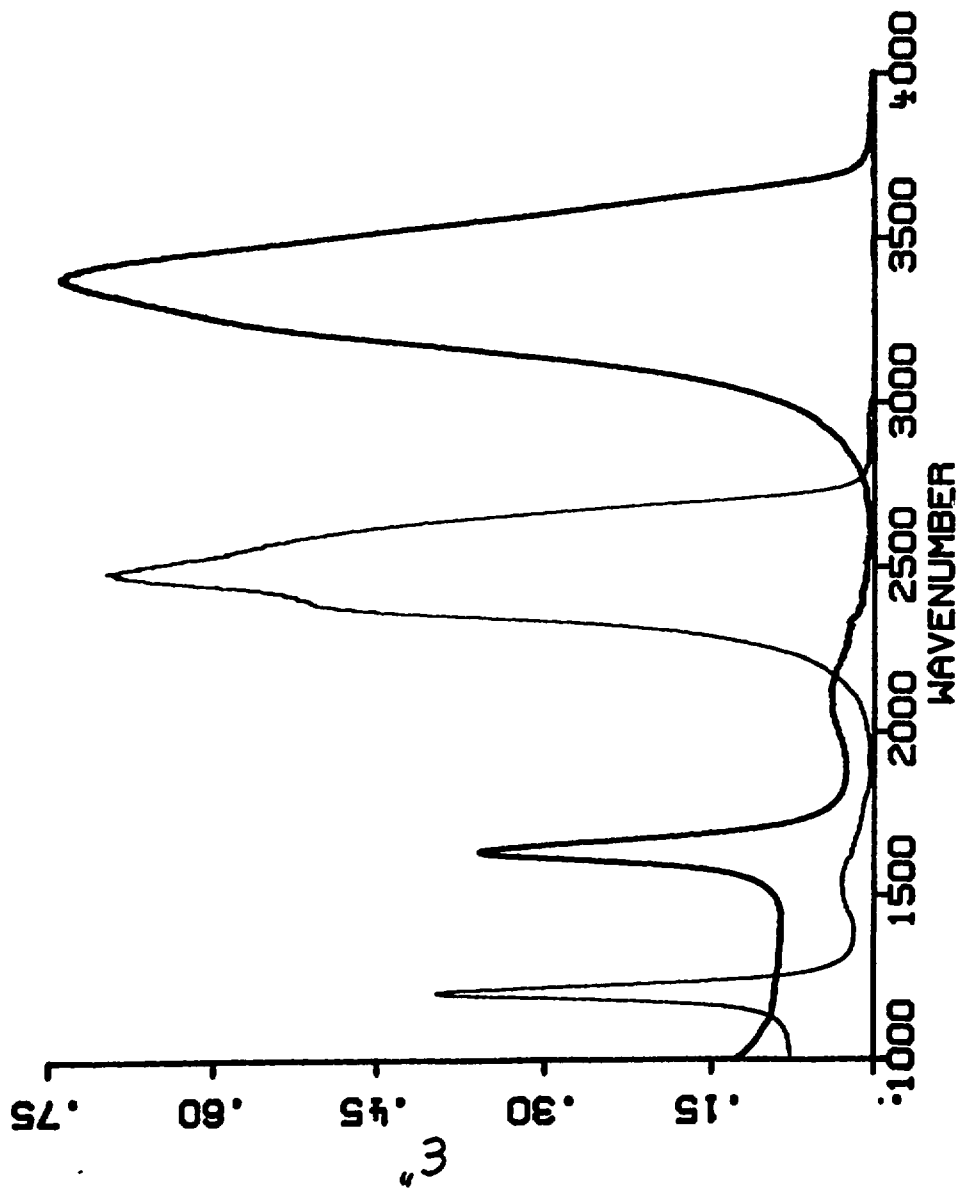


Fig. 1

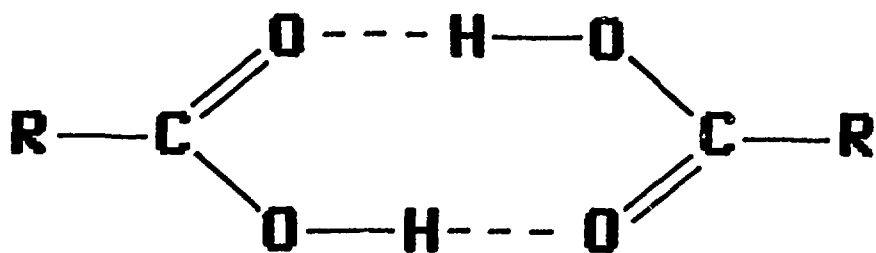
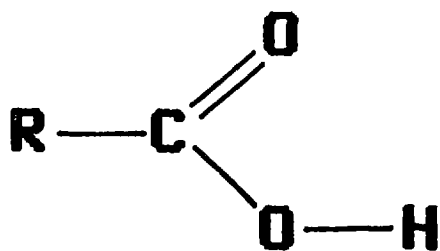


Fig. 2

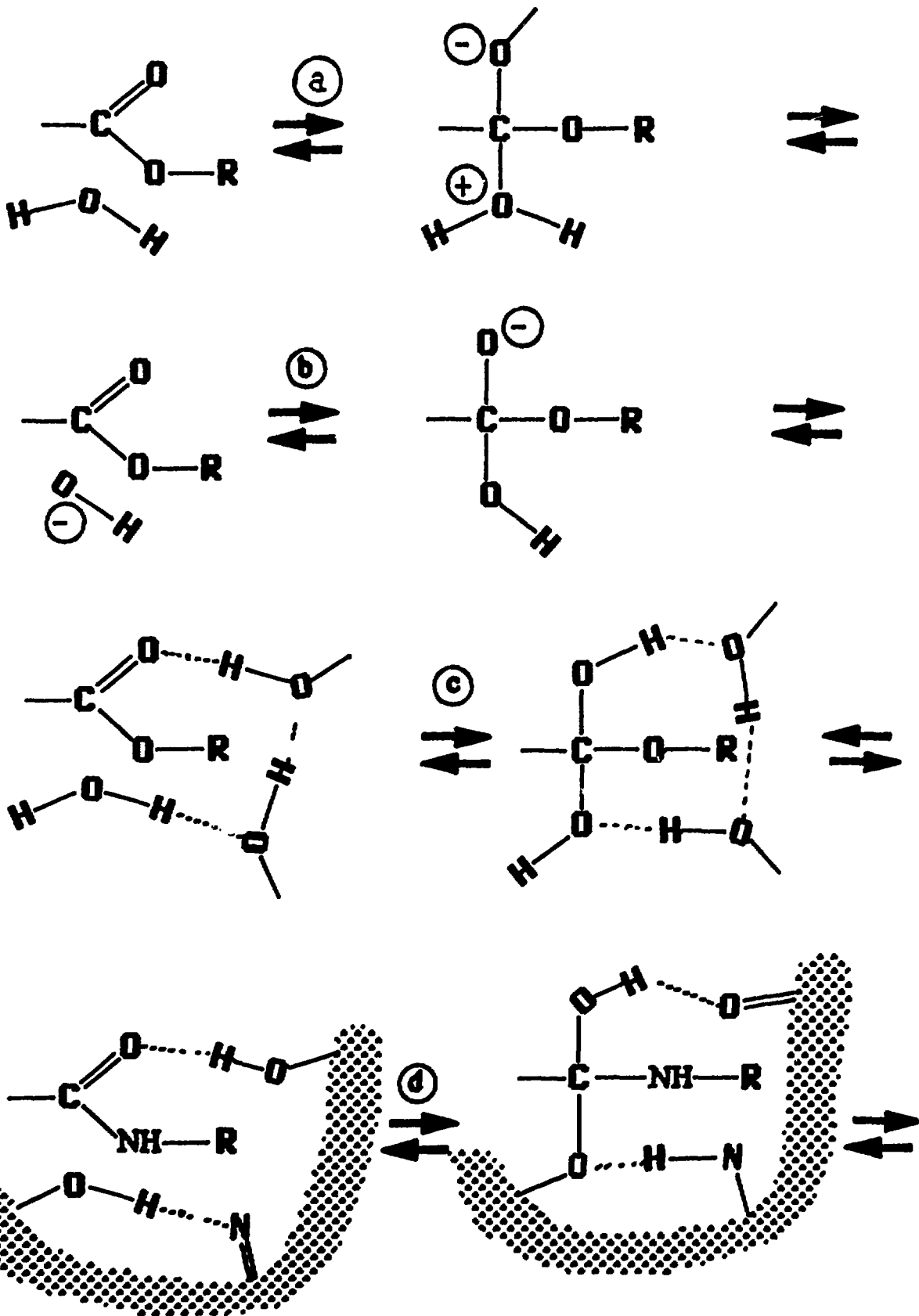


Fig. 4