Proton Dispersion Forces and Continuous Energy Level Distribution of Protons in the Hydrogen Bonds of Semiprotonated Poly-L-histidine and with Model Substances

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IR spectroscopic investigations were conducted with poly-L-histidine (PLH) films, hydrous imidazole and pyrazole solutions and anhydrous N-methylimidazole with respect to protonation. Continuous absorption arises with increasing protonation and is maximally intensive with PLH, imidazole and N-methylimidazole when 50% protonated. The absorption indicates the formation of NH...N bonds. The protons in these bonds are found in continuous energy level distribution. This continuity is due to the extremely large polarizability of symmetrical hydrogen bonds with double minimum potential well in which the proton tunnels. Thus on the one hand proton dispersion forces can arise, and on the other hand the ion fields can exert considerable influence. Both cause an energy level shift. The influence of these tunneling protons on the NH groups in PLH, imidazole and pyrazole is under discussion. The ClO4 ions are added to these NH groups via NH...ClO4 bonds. The CH groups in the N-methylimidazole are so acidic that CH...ClO4 bonds are formed. In the case of PLH, imidazole and N-methylimidazole, the loss of the continuous absorption at more than 50% protonation is explained by the fact that each imidazole ring then possesses a ClO4 ion and thus the NH...N bonds are ruptured. With pyrazole, the continuous absorption continues to increase at 50% protonation. This is due to the fact that its pKa value is considerably less than that of the other substances. Hence H2O2O6 groupings are already formed preferentially before the NH...ClO4 groups. The rise of the continuous absorption in the case of the imidazole solution at a 100% or higher degree of protonation can be explained likewise. The corresponding rise with N-methylimidazole is accounted for by the formation of ClO4H...ClO4 bonds.

Kirkwood and Shumaker postulated forces between protein molecules caused by proton movement. Over the past years, a continuous absorption was observed in the IR spectra of acids, bases and especially of polyelectrolytes with dissociated acidic or basic groups. Beginning at the bands of the OH or OD stretching vibrations, i.e., at about 3500 cm⁻¹ or at about 2600 cm⁻¹ respectively, it extends toward smaller wave numbers. This indicates that the protons are present in continuous energy level distribution, that is, in energy bands, and is closely connected with tunneling protons in hydrogen bonds with a symmetrical double minimum potential well (see Fig. 1 a). The proton can be described by means of two proton boundary structures, as shown in Fig. 1 b. If a proton tunnels in a hydrogen bond isolated from its environment, this indeed results in a splitting of the energy levels.

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![Fig. 1. a) Symmetrical double minimum potential well in a hydrogen bond. b) Proton boundary structures of a hydrogen bond with tunneling proton.](image-url)
levels but never in a continuity of levels. The symmetrical hydrogen bonds with double minimum potential well are, however, extremely polarizable. Therefore the energy levels of the tunneling protons of the hydrogen bonds are shifted, firstly by the coupling between these hydrogen bonds via proton dispersion forces. These arise since the tunneling of the proton is connected with a fluctuation of the electromagnetic field near the hydrogen bond. Neighboring tunneling protons are coupled via these fields. Since the lowest level preferably occupied by the protons is lowered, these forces are attractive forces. A second cause, which can likewise lead to a shift of the tunneling proton energy levels, is the interaction with the Coulomb fields of neighboring anions. In contrast to the forces postulated by Kirkwood and Shumaker, the proton dispersion forces arise not through thermal movement of the protons, but on account of the peculiar characteristics of these hydrogen bonds. The degree of shifting and splitting of the energy levels depends on the distances and orientations of the hydrogen bonds and of those of the anions and the hydrogen bonds. In solution, these distances and orientations have a random statistical distribution. Thus the energy level shifts also have a random statistical distribution, for the coupling between the bonds differs in strength. Hence, a continuous absorption is observed in the IR spectrum (details s. pp. 159 – 214 and *).


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Fig. 2. IR spectra of concentrated hydrous imidazole solutions dependent on % protonation.

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Wavelength [\mu m]

Wave number [cm\(^{-1}\)]
Symmetrical hydrogen bonds with double minimum potential well can form in large number in biological systems in the presence of acceptors with a pKa value of about 7. The nitrogen atom of the imidazole rest in the case of histidine represents such an acceptor.

**Investigation of Model Substances**

**Imidazole**

The imidazole rest** is the functional group of histidine. Therefore we first deal with IR spectra of hydrous imidazole solutions dependent on the protonation. Fig. 2 indicates the formation of a continuous absorption with increasing imidazole protonation. Beginning at the band of the NH stretching vibration, i.e., at about 2500 cm⁻¹ it extends toward smaller wave numbers. Once protonation exceeds 50%, the intensity again decreases at first. Fig. 3 a shows the continuous absorption as a function of protonation in percent. The continuous absorption is at its most intensive when two imidazole rings are cross-linked by one excess proton.

Upon this, NH•••N hydrogen bonds form between two imidazole rings. The continuous absorption indicates that these protons occur in continuous energy level distribution, that is, in energy bands. These NH•••N bonds thus feature the characteristics mentioned above, that is, these bonds are extremely polarizable and between these bonds may act the proton dispersion forces.

The stretching vibration of the imidazole NH groups is observed as an extremely wide, intensive band in the range 3000 – 2500 cm⁻¹. On protonation, this shifts slightly towards smaller wave numbers. The in-plane bending vibration of the NH groups migrates from 1160 cm⁻¹ to 1185 cm⁻¹. Both shifts show that with increasing protonation the imidazole NH groups form somewhat stronger hydrogen bonds. The following are the causes: The ring electrons are attracted by the proton. This, together with the repulsion of both H nuclei, raises the hydrogen bond donator property of the imidazole NH group. These band shifts thus indicate that the charge centre is shifted considerably more during proton tunneling than the H nucleus itself, since the electrons are attracted during proton tunneling in the opposite direction. The Cl⁻ ions are added to these NH groups via hydrogen bonds, since not NH•••N but NH•••Cl⁻ bonds are formed during protonation in compounds in which no acceptor group exists for the Cl⁻ ions, as for instance with pyridine. Fig. 4 a shows the structure at 50% protonation. The NH•••N bonds shall be represented here by the two proton boundary structures shown in Fig. 1 b.

According to CORDES and WALTER, as well as with respect to the paper by ZIMMERMANN, it appears feasible that NH•••N bonds occur even at

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**Fig. 3. Absorbance of the continuous spectrum (evaluated in a spectral range free from water or NH bands) plotted against the protonation in percent. a) Imidazole, b) N-methylimidazole, c) Pyrazole.**

**The assignment of imidazole, methylimidazole and pyrazole bands is given in W. Sessler, private communication.**

13 W. Sessler, private communication.
groupings may be formed by disproportionation. Were this the case, bands characteristic for the protonated imidazole ring, such as the ring vibration at 1588 cm$^{-1}$, for instance, would have to occur already in the spectrum of the 0% protonated solution. This is not the case.

If protonation exceeds 50%, the continuous absorption disappears again, for when an excess proton is added to both imidazole rings, the NH$\cdots$N bond is ruptured. As shown in Fig. 4 a, the NH groups with the Cl$^\ominus$ ions form hydrogen bonds with an asymmetrical potential well. Since the proton cannot tunnel in these bonds, the latter are not as strongly polarizable. As was observed, the continuous absorption must therefore disappear. The NH stretching vibration band in these groups is likewise found in the range 3000 - 2500 cm$^{-1}$ and the NH in-plane bending vibration at 1185 cm$^{-1}$.

$N$-methylimidazole

The formation of the NH$\cdots$Cl$^\ominus$ groups is seen clearly in the case of $N$-methylimidazole. This has two advantages as against imidazole: It can be investigated firstly, as an anhydrous liquid system and secondly, it possesses no NH group.

Fig. 4 shows the $N$-methylimidazole spectra and Fig. 3 b the dependence of the absorbance of the continuous spectrum on the protonation. Here, too, continuous absorption arises with increasing protonation; the intensity decreases again once protonation exceeds 50 percent. That is, NH$\cdots$N bonds form between the rings with $N$-methylimidazole, too. The protons likewise occur in continuous energy level distribution.

Where do the Cl$^\ominus$ ions occur in this system? The CH stretching vibration at 3106 cm$^{-1}$ shifts when protonation rises from 0% to 50% towards smaller wave numbers (3080 cm$^{-1}$), the $\gamma$ CH vibration from 819 cm$^{-1}$ towards larger one (839 cm$^{-1}$) (Fig. 5). Both shifts indicate that these CH groups are so acidic that CH$\cdots$Cl$^\ominus$ hydrogen bonds are formed. However, with increasing protonation NH$\cdots$Cl$^\ominus$ bonds form, even at a slight degree of protonation, in addition to the NH$\cdots$N bonds. This is shown by the wide band of the NH stretching vibration, arising at 2600 cm$^{-1}$, and the NH in-plane bending vibration, arising at 1179 cm$^{-1}$ with increasing protonation. The assignment of these bands is given by comparing the spectrum of the protonated sample with that of the N-deuterated one in Fig. 6. Fig. 7 shows that the number of the NH$\cdots$Cl$^\ominus$ groups at first rises proportionally to the protonation and then — as soon as the continuous absorption disappears, that is, when the NH$\cdots$N bonds are ruptured — rises considerably more steeply.
Fig. 5. IR spectra of anhydrous $N$-methylimidazole dependent on the protonation.

Fig. 6. IR spectra of $N$-methylimidazole. —— $100\%$ $H^\oplus$, ··· ··· $100\%D^\oplus$.

Thus, the structures in Fig. 6 occur with $N$-methylimidazole: the $NH^\oplus\cdots N$ hydrogen bonds shall again be represented by two proton boundary structures as in Fig. 1 b.

**Increase of Continuous Absorption at Protonation Degrees Higher than 100 percent**

The absorbance of the continuous spectrum again rises when protonation exceeds 100% in the case of
Proton dispersion forces and energy level

Fig. 7. N-methylimidazole. —— Absorbance of the continuous spectrum, • • • • Integral absorbance of the NH stretching vibration in the NH\(^{\bullet}\)•••Cl\(^{\bullet}\) group plotted against the protonation.

N-methylimidazole (Figs. 5 and 3 b), as well as with the imidazole solution (Figs. 2 and 3 a). This indicates that other hydrogen bonds now form with the characteristics already mentioned. \(\text{H}_{2}\text{O}_{\bullet}\) groupings, respectively, can now form in the hydrous imidazole solution\(^6,16\). Bonds between water molecules are out of question, however, with N-methylimidazole \(\text{Cl}^{\bullet}\text{H}^{\bullet}\)•••Cl\(^{\bullet}\) bonds evidently form between the Cl\(^{\bullet}\) ions which cause the continuous absorption. Of course, these bonds could also form with the imidazole solution.

Pyrazole

Fig. 8 shows the pyrazole spectra, Fig. 3 c the dependence of the absorbance of the continuous spectrum on protonation. As with the other substances, the absorbance of the continuous spectrum increases in the protonation range 0—50 percent. Here, too, the continuous absorption shows that tunneling protons occur in continuous energy level distribution. NH\(^{\bullet}\)•••N bonds form. These bonds are extremely polarizable and between them may act proton dispersion forces. The band of the stretching vibration of the pyrazole NH group occurs as a very wide band in the range 2800—2300 cm\(^{-1}\). This band in the unprotonated pyrazole lies in the slope of the broad water band, that is, in the range 3200—2600 cm\(^{-1}\), i.e., the NH groups now form far stronger hydrogen bonds. As with imidazole, this indicates that, whilst the proton tunnels, the charge center shifts considerably more strongly than the proton itself, since the proton attracts the electrons in the opposite direction. The second NH group becomes a stronger hydrogen bond donor, due to the above as well as to the repulsion of both H nuclei. This NH band shifts more with pyrazole than with imidazole, due to the fact that the NH group with pyrazole is nearer the excess proton.

The NH in-plane bending vibration lies at 1135 cm\(^{-1}\) and shifts on protonation, contrary to expectation, towards smaller wave numbers, namely to 1128 cm\(^{-1}\). Comparison with the N-deuterated pyrazole spectra in Fig. 9 shows that this band is correctly assigned. The ring vibration \(R_{\delta}\) lies at 1150 cm\(^{-1}\) and on protonation shifts to 1168 cm\(^{-1}\). This shift is caused by a coupling with the NH bending vibration — the latter is pressed by this coupling towards smaller wave numbers — which explains the anomalous behaviour of the NH in-plane bending vibration.

In pyrazole the intensity of the continuous absorption reaches no maximum at 50% protonation but merely rises less steeply (Fig. 3 c). This difference between imidazole and N-methylimidazole on the one hand and pyrazole on the other becomes comprehensible on comparison of the pKa values of

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these substances. The pKa value of imidazole is 6.953\textsuperscript{17} as against a pKa value of 2.5\textsuperscript{18} with pyrazole. Hence the proton is less strongly bonded by the N of the pyrazole and thus H\textsubscript{2}O\textsuperscript{2} groupings form already at protonation degrees smaller than 100 percent. Although the NH\textsuperscript{2}···N bonds are ruptured above 50% protonation, a continuous absorption rise is observed with pyrazole, since the continuous absorption is then caused by the tunneling proton in H\textsubscript{2}O\textsuperscript{2}. 

In summary, the structures occurring with pyrazole are illustrated in Fig. 4 c. The NH\textsuperscript{2}···N bonds are again represented by two proton boundary structures, as in Fig. 1 b.

\textbf{NH\textsuperscript{2}···N Bonds between Imidazole and Pyrazole}

According to BRICKMANN and ZIMMERMANN\textsuperscript{19}, the tunneling frequency depends very sensitively on the symmetry of the double minimum potential well. The same holds good for the polarizability of the hydrogen bonds with double minimum potential well\textsuperscript{9}. If 50% protonated imidazole (pKa 6.95) is mixed with 50% protonated pyrazole (pKa 2.5), one anticipates that NH\textsuperscript{2}···N bonds also form between unlike partners, i.e., bonds with an asymmetrical double minimum potential well. These bonds should therefore not contribute to the continuous absorption since the polarizability of these bonds is only small. The continuous absorption of this mixture is indeed less intensive than that of the purely 50% protonated solution.

\textbf{Poly-L-histidine}

Fig. 10 shows poly-L-histidine spectra dependent on the degree of protonation. In this case, too, a continuous absorption occurs with increasing protonation. The intensity of this continuous absorption decreases again at over 50% protonation with increasing protonation. This continuous absorption is independent of the degree of hydration, as shown in Fig. 11.

Thus, NH\textsuperscript{2}···N bonds likewise form with poly-L-histidine at increasing protonation. In the case of


PLH, too, the cause of the level continuity is found in the extremely large polarizability of the hydrogen bonds with double minimum potential well in which the proton tunnels. Between these bonds may act proton dispersion forces, as with the model substances.

The supposition, that with semi-protonated poly-L-histidine the rings can be cross-linked via hydrogen bonds formed by the excess protons, was already expressed earlier by MIYAZAWA. He writes p. 552: "An interesting speculation can be made, that at half protonation (pKa = 5.9) a definite structure, perhaps helical, is attained whereby a proton is equally shared by two imidazole rings on adjacent turns of the helix".

The extremely wide intensive stretching vibration band of the NH groups of the rest in the range 3000 – 2300 cm\(^{-1}\) is hereby shifted only slightly.

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Fig. 10. IR spectra of poly-l-histidine films dependent on the protonation.

Fig. 11. IR spectra of a 50% protonated poly-l-histidine film dependent on the hydration. Film hydrated in equilibrium with 1 — 90% relative humidity, 2 — 11%, 3 — thoroughly dried.

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\(^*\) With respect to the structure change of poly-L-histidine dependent on the protonation see \(^{31}\).

\(^{31}\) J. MUEHLINGHAUS and G. ZUNDEL, Biopolymers 10, 711 (1971).
towards smaller wave numbers. The in-plane bending vibration of these NH groups shows no shift at 1104 cm\(^{-1}\), increasing merely in intensity. These NH groups are accordingly less impaired by bonds again rupture. An asymmetrical potential as observed, the continuous absorption must disappear. The NH stretching vibration band of these groups lies likewise in the range 3000 — 2300 cm\(^{-1}\). The in-plane bending vibration of these groups appears somewhat shifted towards larger wave numbers at 1145 cm\(^{-1}\), whereas the band at 1104 cm\(^{-1}\) disappears. The NH out-of-plane bending vibration occurs at 953 cm\(^{-1}\), whereas the ring vibration \(R_2\), found at low degrees of protonation at 942 cm\(^{-1}\), shifts to 919 cm\(^{-1}\).

Are such NH\(^{\circ} \cdots \mathrm{N}\) hydrogen bonds with tunneling proton met with in biological phenomena? Which role can the special characteristics of these bonds play in biological phenomena? According to NMR investigations it is probable that histidine shows a marked stacking effect precisely in the case of semiprotonation\(^{22}\), i.e., the histidines are stacked above each other. This is indeed, too, the result of the formation of NH\(^{\circ} \cdots \mathrm{N}\) bonds and the then occurring proton dispersion forces. — Proteins also yield such NH\(^{\circ} \cdots \mathrm{N}\) bonds between histidine rests, for instance in the active centre of ribonuclease such a hydrogen bond is found\(^{23}\). It is conceivable that such hydrogen bonds are significant as far as allosteric processes with proteins are concerned. — ALDRIDGE and ROSE\(^{24}\) have discussed an excess proton conductivity mechanism via hydrogen bonds between imidazole rests of histidine, which should be of significance for the oxydative phosphorylation.

### Experimental Procedures

The poly-L-histidine was obtained from Miles Laboratories Inc., Elkhart, Indiana, USA. According to the charge, this had a mean molecular weight between 8,000 and 16,000. We cleaned this substance by means of electrodialysis. The PLH was subsequently dissolved in 0.1 \(\text{N} \text{HCl}\). The HCl amount was chosen so as to correspond to the desired degree of protonation. The films were dried on germanium dishes in teflon frames. In order to prevent loss by absorption, this must be n-dotted (specific resistance 50 \(\Omega \text{cm}\)). Since PLH is only soluble when protons are present, the unprotonated films were produced from 25% or 50% protonated films by neutralizing with KOH. The KCl was then washed away. The 75% and 100% protonated films show a tendency to light scattering. This disappears when the films are left for some days at 100% humidity. In addition, and in contrast to the very mechanically stable PLH 50% films, these are of exceedingly low mechanical stability. For details of the production of the films refer to\(^{11}\).

As described in\(^2\), p. 259 ff and\(^{25}\), these films are examined in cells dependent on the degree of hydration.

Imidazole was obtained from Merck AG, Darmstadt, and \(N\)-methylimidazole and pyrazole from Fluka AG. The \(N\)-methylimidazole was dried above a molecular sieve and imidazole and pyrazole in the high vacuum at a raised temperature. Pyrazole-d\(_4\) was obtained on adding D\(_2\)O several times to pyrazole and drying. The nonprotonated hydrous imidazole solution contained 720.8 mg/ml, the pyrazole solution 469.2 mg/ml. The

\(^{22}\) H. RUETERJANS, private communication.

\(^{23}\) H. RUETERJANS and H. WITZEL, European J. Biochem. 9, 118 [1969].

\(^{24}\) W. N. ALDRIDGE and M. S. ROSE, FEBS letters 4, 61 [1969].

solutions are protonated with HCl gas. DCI gas was produced from D20 and PCl3, the latter, Suprapur, supplied by Merck AG, Darmstadt. The increase in volume on protonation was determined and taken into consideration when adjusting the layer thickness of the cell, so that the same amount of the substance was constantly subjected to radiation. A layer thickness of 20 μ was taken as a basis for the nonprotonated samples.

The solutions were examined in an IR-cell with variable thickness of the film. This cell was improved as against the usual type in the following way: The cell windows consist of germanium. The cell is completely lined with platinum, thus eliminating any acid corrosion. A vernier with 51 divisions permits a precise adjustment for the thickness of the film. A teflon O-ring with a groove ensures that the cell is well sealed. The cell is fitted outside with a channel all round which allows thermostatization of the cell by circulating water. All spectra are for 30 °C.

The measurements were effected with the Perkin Elmer IR double beam spectrophotometer model 221. On account of the high loss of reflection of the germanium, slit program 980 was used. Registering speed amounted to one wave number per second. The sensitivity was checked during plotting of the spectra. In order to eliminate loss of energy through absorption of the water vapor in the air, the spectrophotometer was flushed with dry air. The spectra drawings are corrected by a calibration curve plotted with the help of the values given in 26.

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Studies of the Substituent Effect in Aromatic Systems
by Chlorine Nuclear Quadrupole Resonance (35Cl-NQR).
Chloroanilinium Salts, \([\text{Cl}_x\text{C}_6\text{H}_{5-x}\text{NH}_3\text{X}^\circ]\) with \(X^\circ=\text{Cl}^\circ, \text{Br}^\circ, \text{etc.}\)

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The 35Cl-NQR spectra of 27 chloroanilinium salts, \([\text{Cl}_x\text{C}_6\text{H}_{5-x}\text{NH}_3\text{X}^\circ]\), mainly chlorides and bromides, were investigated at 77 °K. The 35Cl-NQR frequencies were tentatively assigned to certain chlorine atoms at the benzene ring. The substituent influence of the \(-\text{NH}_3\text{X}^\circ\) group is discussed in terms of the NQR substituent parameter \(\kappa\). The electron attracting power of this group is clearly revealed. It decreases in the order ortho > meta > para of the substitution at the benzene ring with regard to the chlorine position. The substituent parameters \(\kappa\) and \(\sigma\) (Hammett) are compared for the \(-\text{NH}_3\text{X}^\circ\) group and the \(-\text{NH}_2\) group. The anion in the anilinium salts, \(X^\circ\), seems to have no influence on the charge distribution within the aromatic system.

In the study of the influence of substituents on aromatic systems the interest is directed to the \(\text{NH}_3\)\(^\circ\) group for two reasons. The interaction between substituent and aromatic system can be considered as purely inductive since in this substituent no unshared pair of electrons is available to mesomeric interactions with the delocalized \(\pi\)-electrons of the benzene ring. Furthermore, many reaction conditions produce ammonium ions in equilibrium with the parent bases and it seems to be worthwhile to contrast the electronic properties of both species 1.

Nuclear quadrupole resonance (NQR) has been proved to be a valuable tool investigation substituent effects 2—4. The NQR frequency depends on the electric field gradient at the resonating nucleus. Therefore nuclei which exhibit a nuclear quadrupole moment may serve as a sensitive detector to variations in the charge distribution within molecules. Considering 35Cl-NQR spectra of chlorobenzene derivatives BIEDENKAPP and WEISS 4a expressed the NQR

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1 For general reference see e. g.: G. CHUCHANI, Directing and activating effects, in: S. PATAI (ed.), The Chemistry of the Amino Group, Interscience Publ., New York 1968.