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PROTON TRANSLOCATION IN HYDROGEN BONDS WITH LARGE PROTON POLARIZABILITY FORMED BETWEEN A SCHIFF BASE AND PHENOLS.

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SUMMARY:
$OH\cdots N \rightleftharpoons O^{-}\cdots H^{+}N$ hydrogen bonds formed between N-all-trans-retinylidene butylamine (Schiff base) and phenols (1:1) are studied by IR spectroscopy. It is shown that both proton limiting structures of these hydrogen bonds have the same weight with ΔpK_a (50%) = (pK_a protonated Schiff base minus pK_a phenol) = 5.5. With the largely symmetrical systems, continua demonstrate that these hydrogen bonds show great proton polarizability. In the Schiff base + tyrosine system in a non-polar solvent the residence time of the proton at the tyrosine residue is much larger than that at the Schiff base. In CH_2CCl_2 these hydrogen bonds show, however, still proton polarizability, i.e., the position of the proton transfer equilibrium $OH\cdots N \rightleftharpoons O^{-}\cdots H^{+}N$ is shifted to and fro as function of the nature of the environment of this hydrogen bond. Consequences regarding bacteriorhodopsin are discussed.

INTRODUCTION

N-all-trans-retinylidene butylamine - p-cresol complexes were studied by Kristoforov, Zvonkova and Evstigneeva (1) using IR and visible spectroscopy. They found that Schiff base - p-cresol complexes are formed, whereby in the 1:1 complexes the proton in the hydrogen bond is present at the p-cresol molecule. With very large excess of p-cresol, 1:2 complexes are formed and the proton in the (I) $OH\cdots N \rightleftharpoons O^{-}\cdots H^{+}N$ (II) bonds shifts to the base. From this result they suggested (1) that with the protonation of the chromophoric group of rhodopsin two proton donor groups must take part.

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We have shown (2)-(7) that hydrogen bonds of the type (I) $B_1H\cdots B_2 \rightleftharpoons B_1^-\cdots H^+B_2$ (II), in which both proton limiting structures (I) and (II) have noticeable weight, show very large proton polarizabilities. Thus, in the case of such hydrogen bonds the proton may be shifted by their local environments to one or to the other side of the hydrogen bond.

Recently investigating the dark equilibria between different chromophores of bacteriorhodopsin, Fischer and Oesterhelt (8) proposed, as earlier postulated by Blatz, Mohler and Navangul (9), that the absorption spectrum of the retinyl Schiff base is modulated by the interaction with an anionic group. Furthermore, they observed (10) pH-changes with bacterioopsin during reconstitution and suggested on the basis of these results that the Schiff base may interact with two protonable amino acid residues, whereby an $NH^+\cdots B^-$ hydrogen bond should be of particular significance. Finally, Oesterhelt and Lemke (11) observed that an equilibrium at the chromophore of bacteriorhodopsin depends on the nitration of the tyr 26 residue, i.e., on a change of the pK_a value of this residue. On the basis of this result they suggest, that this residue participates in the chromophoric system.

Thus, a study on the nature of $OH\cdots N \rightleftharpoons O^-\cdots H^+N$ bonds formed between a Schiff base and phenols seems of particular interest.

RESULTS AND DISCUSSION

N-all-trans-retinylidene butylamine (Schiff base) with various phenols (1:1) were studied as function of the pK_a of the phenol in CCl_4 solutions, using IR spectroscopy. All systems and results are summarized in the table.

Table

Data of Schiff base + Phenol 1:1 Complexes

Schiff base with the following phenols	pK_a	ΔpK_a x)	% complex formation	% proton transfer	K_{PT}	-log K_{PT}
1	2	3	4	5	6	7
protected tyrosine	11.13 xx)	-4.14	95			
phenol	9.92 (14)	-3.93	96	9.2	0.10	+0.99
2,4-dichlorophenol	7.90 (14)	-1.91	86	15.5	0.18	+0.74
2,3,5-trichlorophenol	6.43 (14)	-0.44	96	18.0	0.22	+0.66
pentachlorophenol	4.74 (14)	+1.25	98	27.2	0.37	+0.43
2,4,6-trinitrophenol	0.81 (14)	+5.18	>99	47.4	0.90	+0.045

x) pK_a of the Schiff base 5.99 taken from ref.(12)

xx) pK_a value of tyrosine taken from ref.(13)

The Schiff base - phenol complex formation equilibria (col.4) were determined from the absorbance of the OH stretching vibration of the phenols at 3600 or 3540 cm^{-1} (see fig.3). This band was calibrated from the spectra of solutions of the pure phenols.

The proton-transfer equilibria in the $OH\cdots N \rightleftharpoons O^{-}\cdots H^{+}N$ hydrogen bonds in these complexes are determined from the integrated absorbance of the C=N stretching vibration of the protonated Schiff base observed at 1655 cm^{-1}. This band is shown for the various complexes in fig.1b-1f, indicated by an arrow. The integrated absorbance of this band was calibrated using the integrated absorbance of the Schiff base - HCl system, fig.1g, in which 100% transfer of the proton to the Schiff base occurs. The percentage proton transfer to the Schiff base is given in col.5 and the equilibrium constant in col.6. These values are plotted as function of the ΔpK_a, i.e. pK_a of the protonated Schiff base minus pK_a of the phenols. The pK_a of the Schiff base is taken from ref.(12) amounting to 5.99, and

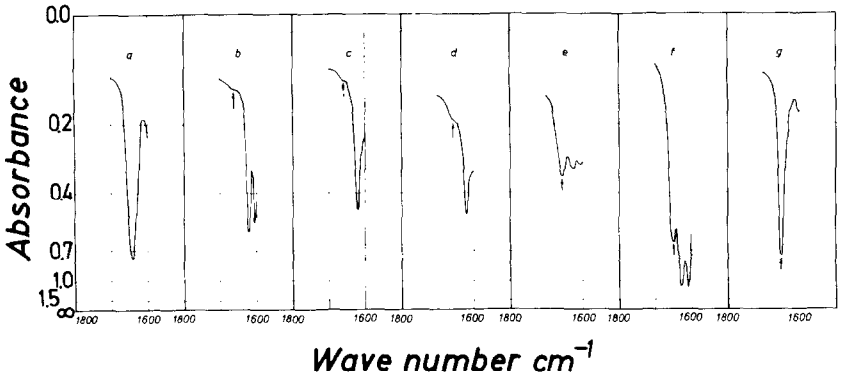

Fig. 1 ν C=N band of N-all trans retinalidine butylamine when a proton is present at the N atom (band indicated with an arrow).

a) Schiff base + protected tyrosine (1:1) (ν C=N band masked by the intense band of the protecting groups, b) Schiff base + phenol (1:1), c) Schiff base + 2,4-dichlorophenol (1:1), d) Schiff base + 2,3,5-tricholophenol (1:1), e) Schiff base + pentachlorophenol (1:1), f) Schiff base + 2,4,6-trinitrophenol (1:1), g) Schiff base + HCl (1:1).

the pK_a values of the phenols given in col.2 are taken from ref.(14). In fig.2a the percentage proton transfer (PT) is plotted as function of the ΔpK_a, and in fig.2b, according to Huyskens (15), -log K_{PT} as function of the ΔpK_a. Both plots show that with the Schiff base + phenol systems in CCl_4 50% transfer of the proton to the Schiff base occurs at $\Delta pK_a^{50\%} = 5.5$.

In the IR spectra of the Schiff base + 2,3,5 trichlorophenol and of the Schiff base + pentachlorophenol system in fig.3b and 3c a comparison of the spectra of the complexes with those of the pure phenols or the pure Schiff base shows that IR continua occur. These continua begin with a band-like structure in the region 3000 - 2500 cm^{-1} and extend towards smaller wave numbers. This spectral feature is particularly characteristic for phenol - amine hydrogen bonds (6). These continua demonstrate (2)-(6) that the OH···N $\rightleftarrows$ $O^-\cdots H^+N$ bonds in these systems show large proton polarizabilities.

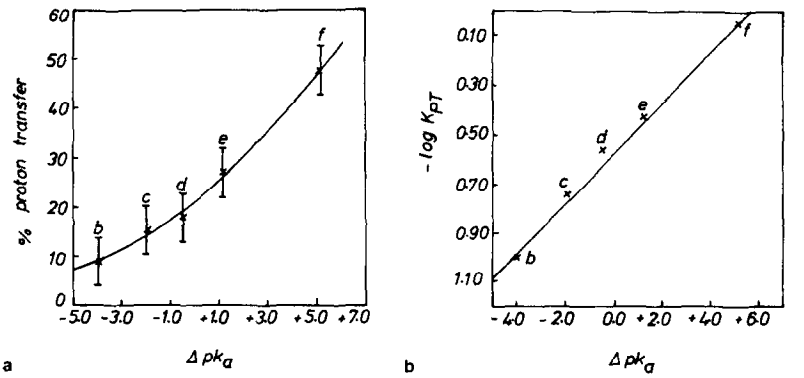

Fig. 2 (c)-(f) numbers of the systems in fig. 1 .

a) Percentage proton transfer as function of the ΔpK_a (pK_a protonated base - pK_a phenol)

b) log K_{PT} (proton transfer equilibrium constant) as function of the ΔpK_a

This result is in good agreement with the fact that both proton limiting structures of these hydrogen bonds have noticeable weight, i.e., with these systems the degree of asymmetry is not very large and the protons fluctuate in these hydrogen bonds.

Fig.3a shows the spectrum of the Schiff base + protected tyrosine (N-acetyl-L-tyrosine ethylester), i.e., tyrosine having protecting groups at its α-carboxylate and α-amino groupings. In this spectrum a continuum is still observed but it is much less intense. This result demonstrates that the $OH\cdots N \rightleftharpoons O^{-}\cdots H^{+}N$ bonds between tyrosine and the Schiff base show still proton polarizability but this polarizability is much smaller than with the systems discussed above. This result is in good agreement with the shape of the proton potentials in tyrosine - Schiff base systems discussed in the following. The position of the proton transfer equilibrium cannot directly be determined in this system since $\nu C{=}N$ is masked by an intense band of the protecting groups (fig.1a). The ΔpK_a value in this sys-

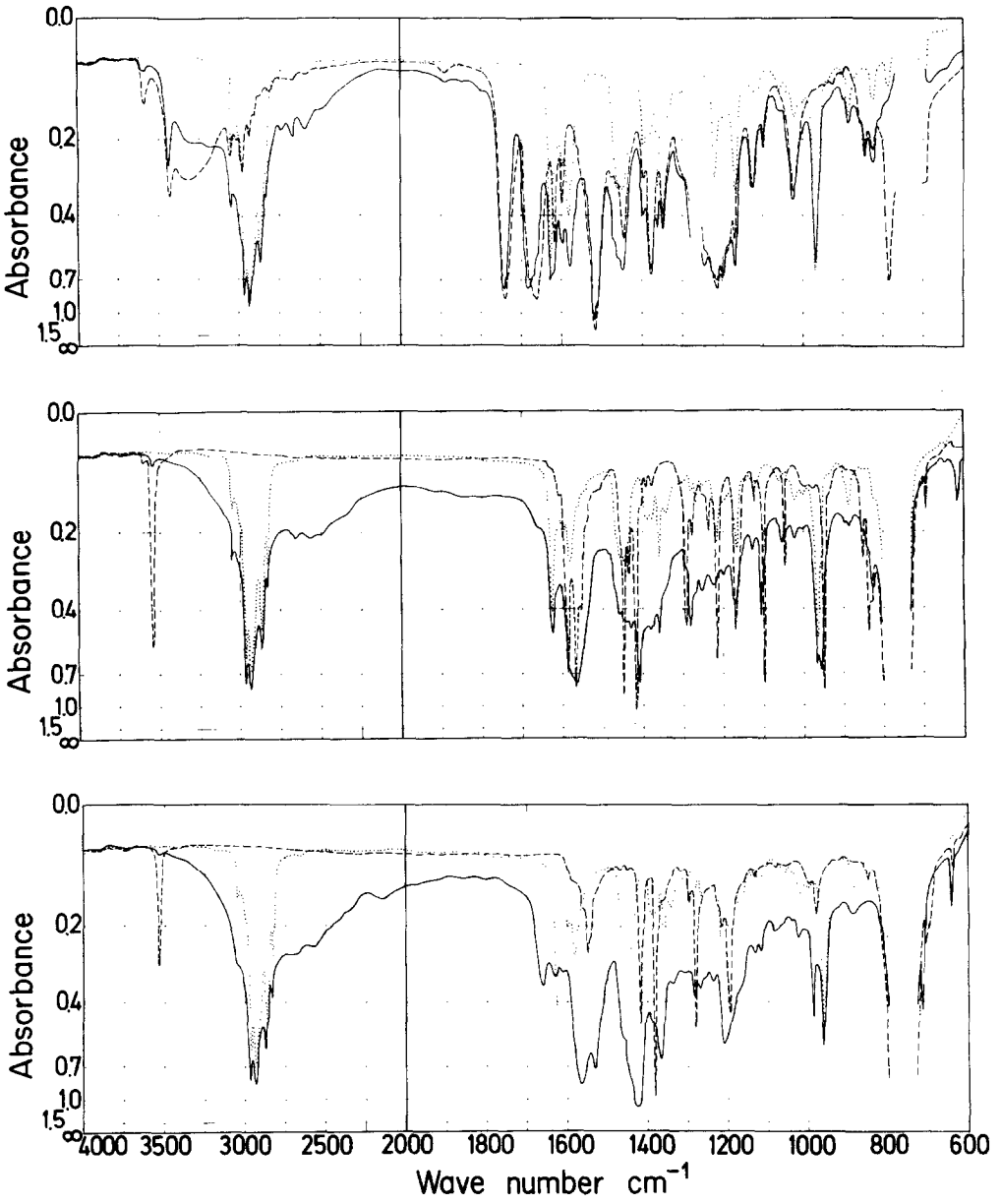

Fig. 3 IR spectra of solutions (layer thickness 50 μm)

a) Solutions in CH_2Cl_2; ··· pure Schiff base (0.5 M), --- pure protected tyrosine (0.5 M), —— Schiff base (0.5 M) + protected tyrosine (0.5 M)

b) Solutions in CCl_4; ··· pure Schiff base (0.5 M), --- pure 2,3,5 - trichlorophenol (0.5 M), —— Schiff base (0.5 M) + 2,3,5 trichlorophenol (0.5 M)

c) Solutions in CCl_4; ··· pure Schiff base (0.5 M), --- pentachlorophenol (0.2 M); —— Schiff base (0.5 M) + pentachlorophenol (0.5 M)

tem, however, amounts to -4.14. Using this value, fig.2a shows that the non-polar proton limiting structure OH···N has much larger weight. Thus, in the 1:1 mixture in CH_2Cl_2 solution the residence time of the proton is much larger at the tyrosine than at the N-atom of the Schiff base.

III. CONCLUSIONS

With retinylidene Schiff base + phenol systems, 1:1 in CCl_4, 50% transfer of the proton occurs at a $\Delta pK_a^{50\%} = 5.5$. The $OH\cdots N \rightleftharpoons O^-\cdots H^+N$ hydrogen bonds found in these systems show large proton polarizability when these systems are largely symmetrical.

With regard to bacteriorhodopsin the Schiff base + tyrosine system is of particular interest. If in this system the $OH\cdots N \rightleftharpoons O^-\cdots H^+N$ bond formed between Schiff base and tyrosine is present in a solvent of low polarity, the residence time of the proton is much larger at the tyrosine residues. This hydrogen bond shows, however, still proton polarizability. Due to this property, the position of this equilibrium is influenced by the polarity of the local environments. Thus, due to the local fields, the proton in this hydrogen bond can be shifted to the tyrosine residue as to the Schiff base.

This explains the result of Kristoforov et al.(1) that in the Schiff base + p-cresol system additional p-cresol shifts the $OH\cdots N \rightleftharpoons O^-\cdots H^+N$ equilibrium in favor of the polar proton limiting structure $O^-\cdots H^+N$, since it is known from many publications (5)(6) (16)-(19) that in hydrogen bonds with great proton polarizability caused by polar environments (in ref.(1) addition of large amounts of p-cresol) the equilibria are shifted in favor of the polar structure $B_1^-\cdots H^+B_2$.

Oesterhelt and Lemke (11) have shown that with bacteriorhodopsin the residue tyr 26 may be involved in the chromophoric structure. Our molecular model built according to the structure of bacteriorhodopsin evaluated by Ovchinnikov et al.(20) may suggest the hypothesis that this tyrosine residue becomes deprotonated in the first step of the photocycle. Our results show that this tyrosine residue could be reprotonated by the protonated Schiff base when the intermediate M_{412} is formed, since, when the environment of this hydrogen bond is not too polar, the residence time of the proton in Schiff base - tyrosine hydrogen bonds is much greater at the tyrosine residue than at the Schiff base.

EXPERIMENTAL PART

N-all-trans-retinylidene butylamine was prepared according to the procedure given in ref.(1). Retinal, as well as the protected tyrosine, i.e., N-acetyl-L-tyrosine ethylester were purchased from Serva, Heidelberg, West Germany.

IR-measurements: For all measurements a cell with sodium chloride windows with 50 µm layer thickness was used. The IR bands of the solvents were compensated by a cell with adjustable layer thickness in the reference beam. The measurements were performed with a Perkin Elmer spectrophotometer, model 325, Bodenseewerk Perkin-Elmer, Überlingen, West Germany.

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REFERENCES

1. V. L. Kristoforov, E. N. Zvonkova and R. P. Evstigneeva, Zh. Obshchei Khim. 44, 909-913 (1974).

2. E. G. Weidemann and G. Zundel, Z. Naturforschung 25a, 627-634 (1970).

3. R. Janoschek, E. G. Weidemann, H. Pfeiffer and G. Zundel, J. Amer. Chem. Soc. 94, 2387-2396 (1972).

4. G. Zundel, in "The Hydrogen Bond - Recent Developments in Theory and Experiments", P. Schuster, G. Zundel und C. Sandorfy eds., Vol. II, North Holland Publ. Co., Amsterdam 1976, pp. 683-766.

5. R. Lindemann and G. Zundel, J. Chem. Soc., Faraday Trans. II 73, 788-803 (1977).

6. G. Zundel and A. Nagyrevi, J. Phys. Chem. 82, 685-689 (1978).

7. W. Kristof and G. Zundel, Biophys. Struct. Mechanism. 6, 209-225 (1980).

8. U. Ch. Fischer and D. Oesterhelt, Biophys. J. 28, 211-230 (1979).

9. P. E. Blatz, J. H. Mohler and H. V. Navangul, Biochemistry 11, 848-855 (1972).

10. U. Ch. Fischer and D. Oesterhelt, Biophys. J. 31, 139-146 (1980).

11. D. Oesterhelt and H.-D. Lemke, J. Europ. Chem., in press.

12. J. J. H. H. M. De Pont, F. J. M. Daemen and S. L. Bonting, Arch. Biochem. Biophys. 140, 267-274 (1970).

13. J. T. Edsall, R. B. Martin, B. R. Hollinworth, Proc. Natl. Acad. Sci. USA 44, 505-518 (1958).

14. J. Drahonovski and Z. Vacek, Collect. Czech. Chem. Comm. 36, 3431-3440 (1971).

15. P. Huyskens and Th. Zeegers-Huyskens, J. Chim. Phys. 61, 81-86 (1964).

16. R. Lindemann and G. Zundel, Biopolymers 16, 2407-2418 (1977), 17, 1285-1304 (1978).

17. J. Jadżyn and J. Małecki, Acta Phys. Polon. A41, 599-616 (1972).

18. M. Schreiber, A. Koll and L. Sobczyk, Bull. Acad. Polon. Sci. Ser. Sci. Chim. 26, 651-654 (1978).

19. J. Fritsch and G. Zundel, J. Phys. Chem. (1981).

20. Yu. A. Ovchinnikov, N. G. Abdulaev, M. Ju. Feigina, A. V. Kiselev and N. A. Lobanov, FEBS Lett. 100, 219-224 (1979).