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# A Model Molecule of the Hydrogen-Bonded Chain in the Active Site of Bacteriorhodopsin

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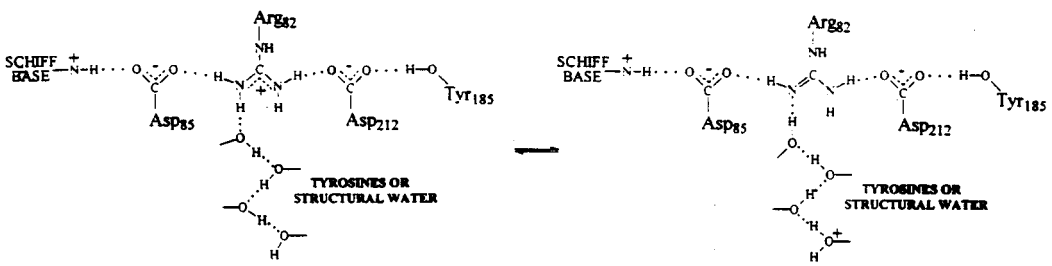
We synthesized a 2-N-methylaminoethyl-tetramethylquanidine amide of Kemp's triacid (CP2). Furthermore, we added to CP2 tetrabutylammonium 4-*tert*-butylphenolate. The $pK_a$ value of 4-*tert*-butylphenol is comparable to that of tyrosine. We studied by FT-IR spectroscopy CP2 and the complex of CP2 with 4-*tert*-butylphenolate. This complex is a model for the hydrogen-bonded chain in the active centre of the bacteriorhodopsin molecule. An intense continuum in the FT-IR spectra demonstrates that this hydrogen-bonded chain shows large proton polarizability due to collective proton motion. As earlier demonstrated also Schiff base carboxylic acid bonds show large proton polarizability. Thus, in all these hydrogen bonds protons can easily be shifted by collective proton motion due to changes of the local electrical fields and by changes of specific interactions arising from conformational changes. 

Recently we studied by FT-IR spectroscopy difference spectra of the intermediates of the photocycle of the bacteriorhodopsin molecule present in the purple membrane (1). On the basis of these results, model building (2) and literature (3)–(7) data, we postulated a pathway for the proton conduction from the active site to the external surface of the membrane as well as a proton pumping mechanism (1). For this pathway and the pumping mechanism, hydrogen bonds (8)(9) and hydrogen-bonded systems with large proton polarizability (9) are of decisive importance. These systems with large proton polarizabilities are indicated by continua in the IR spectra (8)(9). In the meantime we studied models with large proton polarizability for this pathway which is present in the $L_{550}$ intermediate (10)(11).

In ref. 1 we have postulated the following proton limiting structures of the proton pathway we presented in the L 550 intermediate (scheme 1):

In the L 550 intermediate the proton can, however, not be removed from this hydrogen-bonded chain since the proton potential at Arg 82 is too low. It is, however, very well known from FT-IR studies [Siebert et al. (4) and Gerwert et al. (5)] that in the L 550 $\rightarrow$ $M_{412}$ step a proton shifts from the Schiff base to Asp 85 (4)(5), and from respective studies by Rothschild et al. (6)(7) it is known that another proton shifts from Tyr 185 to Asp 212. Other authors have postulated that the latter transfer does not occur. This disagreement in the literature could, however, be caused by the fact that two types of the $M_{412}$ intermediate exist (12)(13). Both hydrogen bonds show large proton polarizability and therefore, these protons can easily be shifted due to changes of local electrical




**SCHEME 1.**



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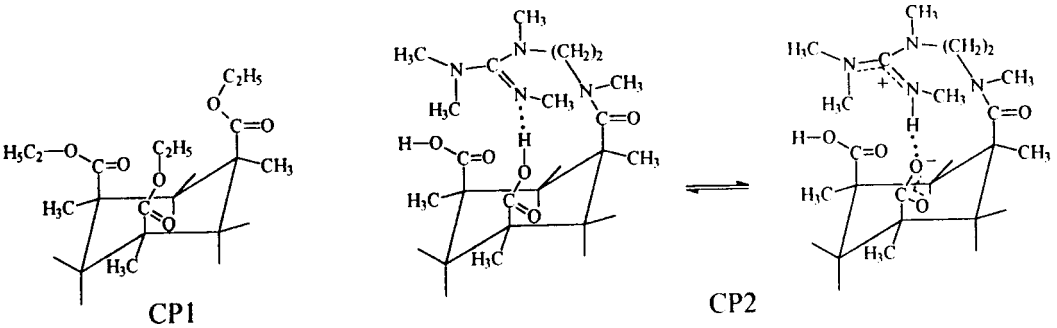



**SCHEME 2.**

fields and specific interactions arising by conformational changes (14)(15). In this way the negative charge in the neighbourhood of Arg 82 is neutralized and the chemical potential of the proton at Arg 82 raised. Thus, the proton can be conducted and removed from the external end of the hydrogen-bonded chain.

## RESULTS AND DISCUSSION

In this paper we studied by FT-IR spectroscopy a model compound (CP2) with a hydrogen-bonded system corresponding to the system $Arg_{82}$–$Asp_{215}$–$Tyr_{185}$ in the active site of bacteriorhodopsin. In scheme 2 a triethylester of Kemp's triacid (CP1) and the model compound (CP2) are shown.

The FT-IR spectra of these molecules are given in Fig. 1. Additionally the spectrum of the CP2 + 4-*tert*-butylphenolate is given. The 4-*tert*-butylphenol has almost the same $pK_a$ value as Tyr.

In the spectrum of the triester no infrared continuous absorption is found. In the spectrum of CP2 an intense IR continuum is observed indicating that the $OH\cdots N \rightleftharpoons .O^{-}\cdots H^{+}N$ hydrogen bonds formed between the carboxylic acid group and the N-atom of the guanidine rest are hydrogen bonds with double minimum proton potential showing large proton polarizability (scheme 2, CP2).

If the 4-*tert*-butylphenolate molecule is added to the solution of CP2, a very intense IR continuum arises indicating that the hydrogen-bonded system shows large proton polarizability due to collective proton motion. Two proton limiting structures of this system are shown in scheme 3.

Thus, the protons can easily be shifted in this systems by changes of local electrical fields or by changes of specific interactions.

The same spectra in the carbonyl region are shown in Fig. 2. In the spectrum of CP2 a very broad band of $\nu$(C=O) with a maximum at 1709 $cm^{-1}$ and a band of $\nu_{as}$ ($CO_2^-$) at 1609 $cm^{-1}$ are observed. The band at 1647 $cm^{-1}$ is the Amide I band.

In the spectrum of the 1:1 complex of CP2 with 4-*tert*-butylphenolate the $\nu$(C=O) band shifts

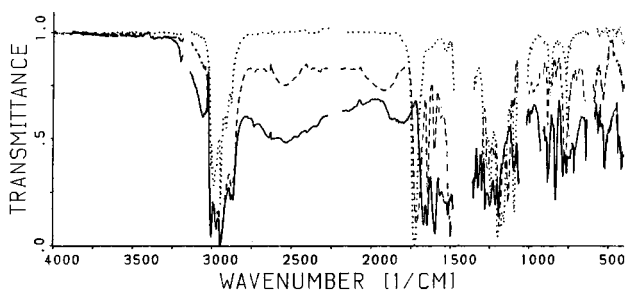
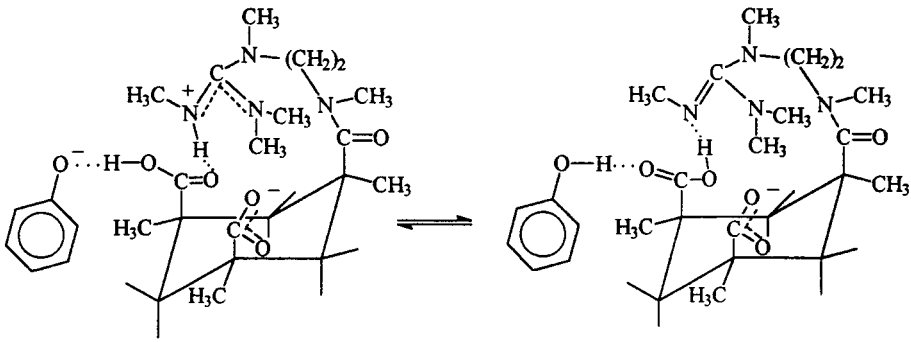



**FIG. 1.** FT-IR spectra of chloroform–acetonitrile (ratio 3:1) solutions of: (– – –) CP2 and (——) its 1:1 complex with 4-*tert*-butylphenol. For comparison, the spectrum of CP1 (· · ·) is given.



**SCHEME 3.**

to 1670 $cm^{-1}$ and the $\nu_{as}$ ($CO_{2^-}$) band is found at 1597 $cm^{-1}$. The Amide I band is observed at 1647 $cm^{-1}$. All these results confirm the formation of the hydrogen-bonded chain shown in scheme 3.

## CONCLUSIONS

As shown earlier (14) the Schiff base carboxylic acid hydrogen bond is also a hydrogen bond with large proton polarizability and thus, the proton can be shifted in this bond by changes of local fields or specific interactions due to conformational changes. In this study it is demonstrated that hydrogen-bonded chains formed in the 1:1 complex of CP2 with 4-*tert*-butylphenolate show large proton polarizability due to collective proton motion. Thus, in these chains the protons can also easily be shifted by changes of the local electrical fields or by changes of specific interactions. This result proves that the protons in the active site of bacteriorhodopsin can also be collectively shifted in the $Tyr_{185}$, $Asp_{212}$, $Arg_{82}$ hydrogen-bonded chain.

## EXPERIMENTAL PROCEDURES

CP2 is analogously synthesized as described in (16), whereby instead of N-methylaminoethylguanidine sulfate the N-methylaminoethyl-tetramethyl-guanidine sulfate was used.

The complex of CP2 with tetrabutylammonium 4-*tert*-butylphenolate was prepared by adding the respective amounts of a 0.1 mol $dm^{-3}$ acetonitrile solution of tetrabutylammonium 4-*tert*-butylphenolate to the solution of CP2 in acetonitrile. The solvent was removed under reduced pressure and the residue was dissolved in chloroform-acetonitrile (ratio 3:1).

The IR spectra were taken in 3:1 chloroform-acetonitrile solutions (0.1 mol/$dm^3$) with a FT-IR spectrometer, Bruker IFS 113v, using a cell with Si windows (sample thickness 0.176 mm, detector DTGS, resolutions 2 $cm^{-1}$).

All solvents were stored over 3Å molecular sieves. All preparations and transfers of solutions were carried out in a carefully dried glovebox under nitrogen atmosphere.

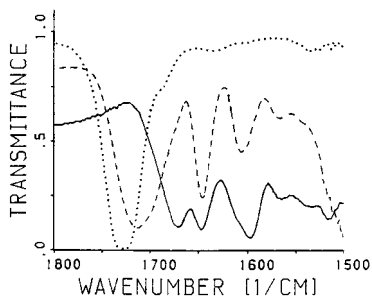



**FIG. 2.** FT-IR spectra of 3:1 chloroform–acetonitrile solutions of: (– – –) CP2 and (——) its 1:1 complex with 4-*tert*-butylphenol in the region of the carbonyl bands. For comparison the spectrum of CP1 (· · ·) is given.

## ACKNOWLEDGMENTS

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