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EASILY POLARIZABLE $N^+H\cdots N$ HYDROGEN BONDS BETWEEN HISTIDINE SIDE CHAINS AND PROTON TRANSLOCATION IN PROTEINS.

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SUMMARY:
Histidinium perchlorate having protecting groups at the α-amino and α-carboxylate group is studied by IR spectroscopy as function of the addition of protected histidine molecules. An intense continuous absorption arises, indicating that the $N^+H\cdots N \rightleftarrows N\cdots H^+N$ formed are easily polarizable hydrogen bonds. From the integral absorbance of a band the concentration of the histidine-histidinium complex, i.e. the concentration of the easily polarizable hydrogen bonds is determined. It is shown that the absorbance of the continuum increases in proportion to the concentration of the easily polarizable $N^+H\cdots N \rightleftarrows N\cdots H^+N$ bonds. Finally, it is discussed that via such an easily polarizable histidine-histidinium hydrogen bond a proton translocation in the active center of ribonuclease A may occur.

INTRODUCTION

The presence of hydrogen bonds showing very large proton polarizabilities is a prerequisite to the translocation of protons within a system via a Grotthus mechanism (1)(2). It was already shown that various homoconjugated $B^+H\cdots B \rightleftarrows B\cdots H^+B$ or $A^-\cdots HA \rightleftarrows AH\cdots {}^-A$ and heteroconjugated $AH\cdots B \rightleftarrows A^-\cdots H^+B$ bonds between side chains in proteins are easily polarizable (3)-(9). Large proton polarizabilities were proved by IR studies with the homoconjugated hydrogen bonds in the case of $N^+H\cdots N \rightleftarrows N\cdots H^+N$ bonds between histidine residues with $(L\text{-his})_n$ (3) and in the case of $OH\cdots O^- \rightleftarrows {}^-O\cdots HO$ bonds with $(L\text{-glu})_n$, $N^+H\cdots N \rightleftarrows N\cdots H^+N$ bonds with $(L\text{-lys})_n$ (9), $SH\cdots S^- \rightleftarrows {}^-S\cdots HS$ bonds with $(L\text{-cys})_n$ and $OH\cdots O^- \rightleftarrows {}^-O\cdots HO$ bonds with $(L\text{-tyr})_n$. The large proton polarizabilities are indi-

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cated in the IR spectra by continuous absorptions (1). With the polymer systems discussed above, sometimes light scattering at the films occurs, hindering quantitative evaluation. Therefore, in this paper $N^{+}H\cdots N \rightleftharpoons N\cdots H^{+}N$ bonds formed between histidine side chains are studied in solutions, using histidine with protecting groups at the α-amino and α-carboxylate groups.

RESULTS AND DISCUSSION

Fig. 1 shows IR spectra of 1M solutions of the protonated protected histidine as function of the addition of protected histidine. This figure shows that with the addition of histidine to the histidinium solutions a continuum arises in the spectra, extending from about 3000 cm^{-1} toward smaller wave numbers on the whole wave number region studied. Continua of this type indicate that the $N^{+}\tilde{H}\cdots N \rightleftharpoons N\cdots H^{+}N$ bonds formed are easily polarizable hydrogen bonds of medium length (10). The band-like doublet structure observed in the region 2700-1900 cm^{-1} is characteristic of hydrogen bonds of this type and is caused by FERMI resonance of the fundamentel transitions in these hydrogen bonds with combinational vibrations (11)-(14).

Fig. 2a and table 1 show that with the addition of histidine to the histidinium solution the absorbance of the continuum below

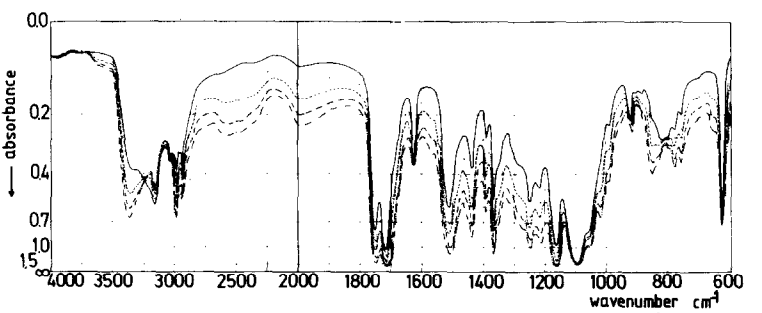

Fig. 1 IR spectra of t-Boc-his-OMe + the corresponding hydroperchlorate in acetonitrile solutions:
——— 0:1·0; ···· 0.25:1·0; ---- 0.50:1·0; -·-·- 1·0:1·0;
—··— 1·5:1 mole dm^{-3}.

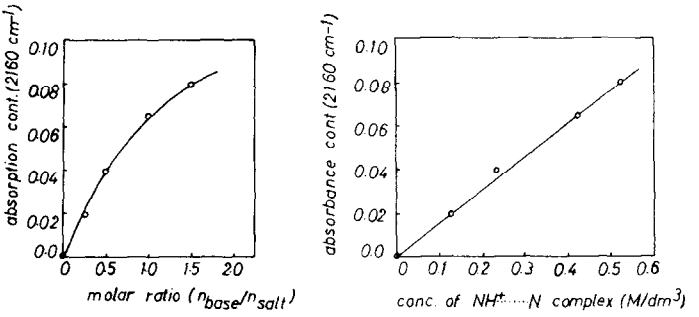

Fig. 2a) Absorbance of the continuum plotted against the various molar ratios of histidine to histidinium perchlorate.

b) Absorbance of the continuum plotted as function of $(NH\cdots N)^+$ complex formed with increasing addition of histidine to a 1·0 M solution of histidinium perchlorate.

a mole ratio of 1 increases less than proportional to the histidine concentration, whereas with addition of an excess amount of free base the intensity of the continuous absorbance further increases. This demonstrates that no complete formation of the histidine-histidinium complexes occurs. The formation of the histidine-histidinium complexes, i.e. the concentration of $N^+H\cdots N \rightleftharpoons N\cdots H^+N$ bonds can be estimated by evaluating characteristic bands in the spectra.

In the region 3500-3100 cm^{-1} the NH stretching vibrations are observed. At 3160 cm^{-1} the NH stretching vibration of the protonated histidine residues decreases with the complex formation, whereas at 3360 cm^{-1} the NH stretching vibration of the NH groups which are not involved in the $N^+H\cdots N \rightleftharpoons N\cdots H^+N$ bonds arises. The latter band is superimposed on the stretching vibration of the amide groups and furthermore, the stretching vibration of histidine molecules not involved with complex formation occurs in the same position. Therefore, the band at 3360 cm^{-1} cannot be used to determine the complex formation.

Table

Data of the Complex Formation between Histidinium and Histidine

molar ratio $c_{base} : c_{base \cdot HClO_4}$ mole/dm^3	absorbance of the band at 1625 cm^{-1}	concentration of $(NHN)^+$ complex in mole/dm^3	equilibrium constant for complex formation: $K = \frac{c}{(c_{base}-c)\ (c_{base \cdot HClO_4}-c)}$
0.0 : 1.00	0.24	0.00	-
0.25 : 1.00	0.21	0.13	1.20
0.50 : 1.00	0.18	0.23	1.17
1.00 : 1.00	0.14	0.43	1.28
1.50 : 1.00	0.11	0.53	1.17

The decrease of the band at 3160 cm^{-1} indicates the complex formation. This band, however, cannot be evaluated very well. Therefore, a ring stretching vibration at 1625 cm^{-1} is evaluated which is caused by the protonated imidazole residues of histidinium and which vanishes with complex formation. From the integral absorbance of this band the concentration of the complex was determined. For the formation of the complex the calculated equilibrium constants K are listed (see tab. last col.), and as a mean value K = 1.20 can be given. The absorbance of the continuum as function of this concentration is plotted in fig. 2b. This figure shows that the absorbance of the continuum increases in proportion to the formation of the easily polarizable $N^{+}H\cdots N \rightleftarrows N\cdots H^{+}N$ hydrogen bonds between the histidinium and histidine residues.

CONCLUSIONS

The IR results show that the $N^{+}H\cdots N \rightleftarrows N\cdots H^{+}N$ bonds formed between histidinium and histidine residues in proteins are easily polarizable hydrogen bonds of medium length. Thus, protons can be translocated via these types of hydrogen bonds and these bonds may be part of hydrogen bonded systems conducting protons via a Grotthus mechanism.

Rüterjans and Witzel (15) have shown by NMR measurements that such histidinium-histidine hydrogen bonds may be present in the active center of ribonuclease A. Thus, it seems possible that a proton translocation via this easily polarizable hydrogen bond occurs with the catalytic mechanism in this enzyme.

EXPERIMENTAL PART

Substances:

The protected amino acid N-tert butoxycarbonyl-l-histidine-O-methyl-ester (t-Boc-his-OMe) was obtained from the Max-Planck-

Institut für Biochemie, Martinsried, W.Germany. The perchlorate-salt of the compound was obtained by dissolving t-Boc-his-OMe in methanol, adding an equimolar amount of an aqueous solution of $HClO_4$ and removing the solvent at reduced pressure.

IR-measurements:

For all measurements acetonitrile was used as solvent at a layer thickness of 15 µm. The windows of the IR cell were made of NaCl. The specific IR bands of the solvent could be compensated by using a cell with adjustable layer thickness in the reference beam. The measurements were performed with a Perkin-Elmer 325 spectrophotometer, Bodenseewerke Perkin-Elmer, Überlingen, W. Germany.

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