Aspartic Acid-Aspartate and Glutamic Acid-Glutamate Hydrogen Bonds Having Great Proton Polarizability – IR Investigations

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α-Amino and α-carboxylate group protected aspartate (Z-asp-OMe) and glutamate (Z-glu-OBZl) solutions in CH_2Cl_2 are studied as function of the addition of the respective salts by IR spectroscopy. The antisymmetrical stretching vibration of the $-CO_2^-$ groups shifts to the position where $\nu\,C=O$ of the acid groups is observed, indicating that both anions in the acid-anion complexes are strongly influenced by the proton. With the formation of these complexes a continuum arises preferentially in the region 1600–900 cm^{-1} demonstrating that the $OH\cdots O^- \rightleftharpoons {}^-O\cdots HO$ bonds formed show great proton polarizability. These hydrogen bonds are short and the proton potential is probably a broad flat well. The degree of the formation of these hydrogen bonds is determined. Finally, it is discussed that positive charge can easily be translocated via such hydrogen bonds in protein molecules.

Introduction

Hydrogen bonds show great proton polarizabilities when double minimum proton potentials or potentials with broad flat wells are present in these hydrogen bonds [1–3]. Via such hydrogen bonds positive charge may be shifted and via chains of such hydrogen bonds positive charge may be conducted by a Grotthus mechanism ([3] p. 761) [4]. It was already shown that various homoconjugated $B^+H\cdots B \rightleftharpoons B\cdots H^+B$ or $A^-\cdots HA \rightleftharpoons AH\cdots A^-$ and heteroconjugated $AH\cdots B \rightleftharpoons A^-\cdots H^+B$ bonds between side chains in proteins show great proton polarizability [5–9]. The large proton polarizabilities are indicated in the IR spectra by intense continuous absorptions [3] and in the Raman spectra by intense Rayleigh wings near the excitation line [10].

In the following using α-amino and α-carboxylate group protected amino acids, the nature of the hydrogen bonds formed between aspartic acid and aspartate and between glutamic acid and glutamate residues are studied.

Results and Discussion

In Fig. 1 a the IR spectra of solutions of protected aspartic acid, of protected aspartate, and of a 1:1 mixture of the acid and the salt are given, whereas Fig. 1 b shows the spectra of aspartate as function of the addition of aspartic acid. These figures show that when aspartic acid is added to the aspartate solution the band of the antisymmetric stretching vibration of the $-CO_2^-$ ions, observed at about 1600 cm^{-1}, vanishes. Simultaneously the intensity of the band complex in the region 1650–1780 cm^{-1} increases. With the acid, to this band complex, $\nu\,C=O$ of the protecting groups, as wall as $\nu\,C=O$ of the acidic $-COOH$ groupings contribute. Thus, these band changes demonstrate that the aspartic acid OH groups form hydrogen bonds with the aspartate residues. In these structurally symmetrical $OH\cdots O^- \rightleftharpoons {}^-O\cdots HO$ bonds the proton influences the electron structure of both aspartate residues strongly and to the same extend, *i.e.*, the CO groups of both residues gain double bond character.

Furthermore, with increasing addition of acid, *i.e.*, with increasing formation of $OH\cdots O^- \rightleftharpoons {}^-O\cdots HO$ hydrogen bonds, a continuous absorption arises which is particularly intense in the region 1600–900 cm^{-1}. This continuum demonstrates that the hydrogen bonds formed show great proton polarizability. The continuum is most intense in the region 1600–900 cm^{-1}. The comparison with the intensity distribution of calculated continua shows [11] that these hydrogen bonds are relatively short hydrogen bonds with large proton polarizability. Probably the proton potential in these hydrogen bonds is a broad flat single minimum.

Similar results are obtained with the solutions of protected glutamic acid + glutamate. To determine

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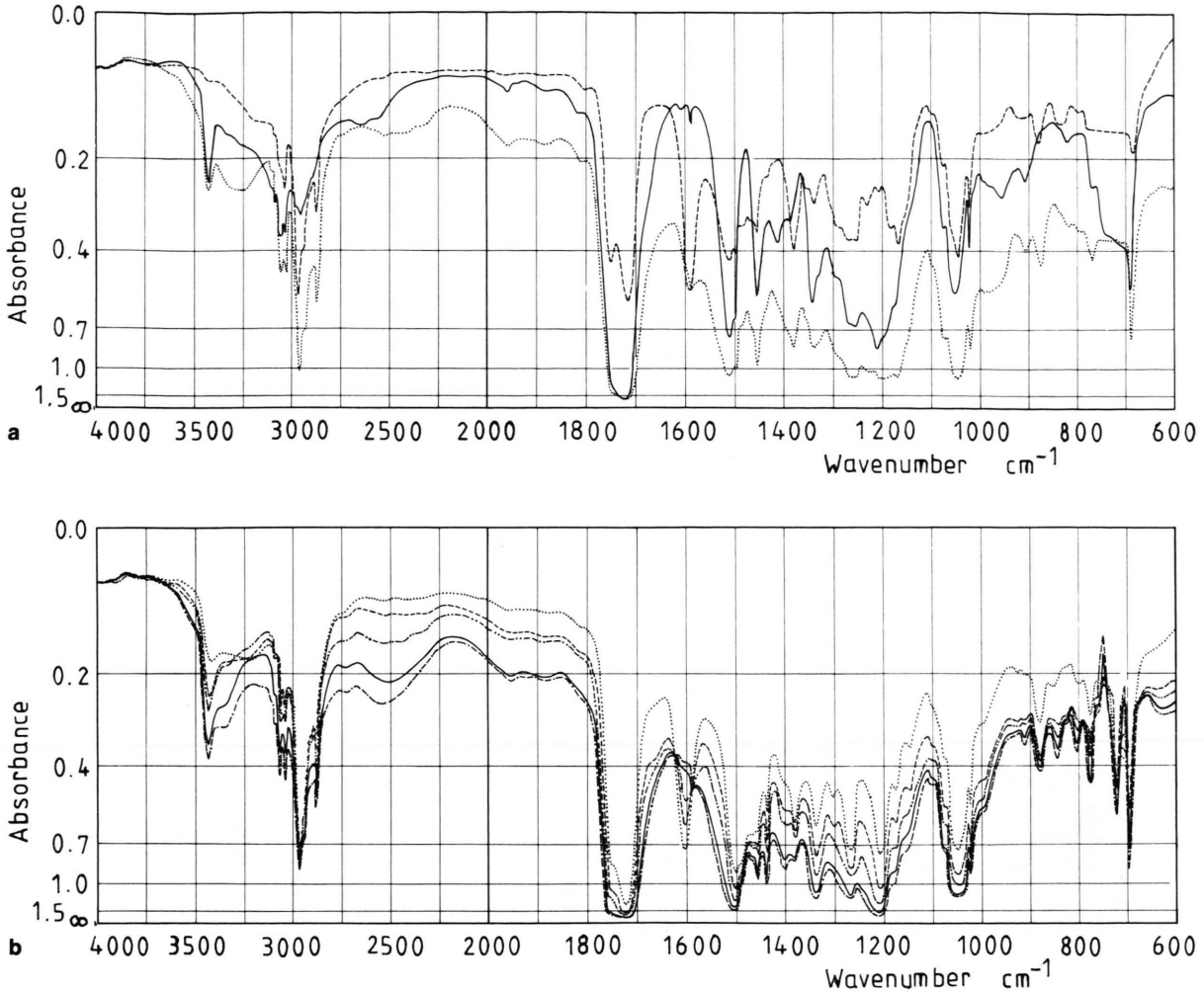

Fig. 1. a. IR spectra of solutions in CH_2Cl_2 at 25 °C: —— protected aspartic acid 1.0 M, layer thickness 25 μ ; - - - - tetrabutylammonium salt of this compound 0.1 M, layer thickness 100 μm; 1:1 mixture of the protected aspartic acid and the respective salt, 1.0 M, layer thickness 25 μm. b. IR spectra of solutions in CH_2Cl_2 at 25 °C: . . . . acid:salt 0.25 M:1 M; - - - - acid:salt 0.5 M:1 M; · · - - · · - - acid salt 0.75:1 M; —— acid:salt, 1 M:1 M; - - - - acid:salt, 2 M:1 M.

the degree of formation of the hydrogen bonds in Fig. 2 the absorbance of the continuum is shown as function of the added acid. This figure demonstrates that complete formation of the hydrogen bonds with great proton polarizability occurs with the aspartic acid-aspartate systems at a mol ratio of about 1.5 mol acid per mol salt, and with glutamic acid-glutamate systems at a mol ratio of about 2.5. In the 1:1 mixtures in the case of the aspartic acid-aspartate systems 95%, and in the case of the glutamic acid-glutamate systems 75% of the hydrogen bonds are formed.

Conclusions

It is shown that the structurally symmetrical hydrogen bonds formed between aspartic acid and aspartate residues and formed between glutamic acid and glutamate residues are short hydrogen bonds with great proton polarizabilities. The proton potential in these hydrogen bonds is probably a broad flat well. Thus via such hydrogen bonds positive charge can be translocated in proteins.

A proton translocation via an aspartic acid-aspartate hydrogen bond may occur in the active

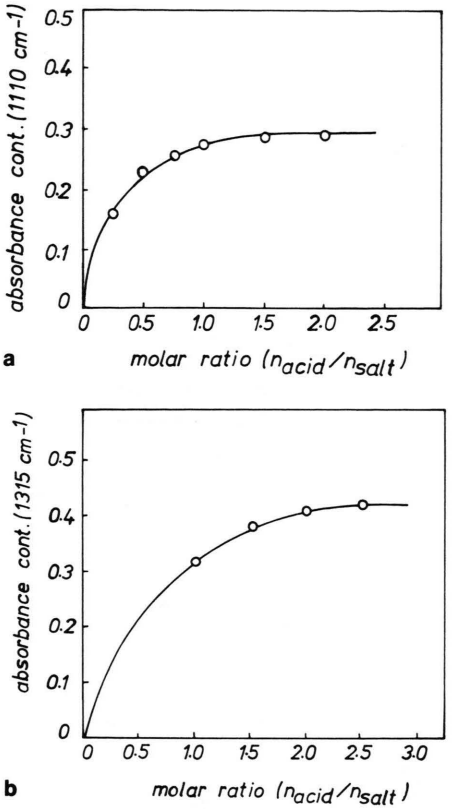

Fig. 2. Absorbance of the IR continuum as a function of the molar ratio n_{acid}/n_{salt}. a. Protected aspartic acid/protected aspartate systems; b. protected glutamic acid/protected glutamate systems.

center of the acidic protease penicillopepsin [12, 13]. In this enzyme the positive charge may be shifted from asp^{32} to asp^{215} and in this way asp^{32} becomes negatively charged and thus reactive. Furthermore, at the inner surface of the purple membrane a large number of aspartate and glutamate residues are present [14]. It was already postulated by Ovchinnikov and coworkers [14] that these groupings mediate the uptake of the protons by the purple membrane. Also with this mechanism hydrogen bonds of the discussed type should be formed.

Experimental

The protected amino acids N-benzyloxycarbonyl-aspartic acid-O-methylester (Z-asp-OMe) and N-benzyloxycarbonyl-glutamic acid-O-benzylester (Z-glu-OBzl) were obtained from the Max-Planck-Institut für Biochemie, Martinsried, Bundesrepublik Deutschland.

Tetrabutylammonium aspartate and glutamate of the corresponding protected amino acids were prepared by dissolving equimolar amounts of 35% tetrabutylammonium hydroxide and Z-asp-OMe or Z-glu-OBzl, respectively in dichloromethane. The excess of solvent was removed by rotatory distillation method. The obtained residues were treated repeatedly (3–4 times) with absolute ethanol to get rid of the last traces of water and finally dried under reduced pressure.

IR measurements: a NaCl cell with varying path length and a Perkin-Elmer spectrophotometer model 325 have been used for recording the IR spectra. Dichloromethane has been used as solvent.

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