Biophys. Struct. Mech. 6, 209–225 (1980)

Biophysics of Structure and Mechanism

Proton Transfer in and Polarizability of Hydrogen Bonds in Proteins. Tyrosine-Lysine and Glutamic Acid-Lysine Hydrogen Bonds – IR Investigations

W. Kristof and G. Zundel

Physikalisch-Chemisches Institut, Universität München,
Theresienstrasse 41, D-8000 München 2, Federal Republic of Germany

Abstract. The $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ hydrogen bonds formed between tyrosine and lysine, and between glutamic acid and lysine residues are studied by infrared spectroscopy considering the following systems: $(L\text{-lys})_n$ + phenol, copoly $(L\text{-lys}, L\text{-tyr})_n$, $(L\text{-lys})_n + (L\text{-tyr})_n$ and $(L\text{-lys})_n + (L\text{-glu})_n$. The phenol-lysine hydrogen bonds are largely symmetrical in the average if the pK_a of the protonated lysine is 2.2 units larger than that of the phenols. In the case of the hydrogen bonds between tyrosine and lysine residues in copoly $(L\text{-lys}, L\text{-tyr})_n$ and $(L\text{-lys})_n + (L\text{-tyr})_n$, the weight of the proton limiting structure $OH \cdots N$ is 80–90%, and that of the polar $O^- \cdots H^+N$ structure 10–20%. Double minimum proton potentials occur but the proton is preferentially present at the tyrosine residues. In the $(L\text{-lys})_n + (L\text{-glu})_n$ system, the protons are present at the lysine residues. Thus, these hydrogen bonds have very large dipole moments (about 10 D). With the lysine-phenole hydrogen bonds, hydration shifts the proton transfer equilibrium a little in favour of the polar proton limiting structure $O^- \cdots H^+N$. These hydrogen bonds are broken to a large extent, however, when only about 3 water molecules are present per lysine residue. When less water is present, as in the copoly $(L\text{-lys}, L\text{-tyr})_n$ and $(L\text{-lys})_n + (L\text{-tyr})_n$ systems, these hydrogen bonds are, however, formed quantitatively. Thus – as discussed in this paper – the tyrosine-lysine hydrogen bonds can participate in proton conducting hydrogen bonded systems – as, for instance, present in bacteriorhodopsin – performing the proton transport through hydrophobic regions of biological membranes.

Key words: Conformation – Polarizable hydrogen bonds – IR spectroscopy – Proteins – Proton transfer

I. Introduction

Infrared studies of $(L\text{-glu})_n$ + N-base (Lindemann and Zundel 1977a) and of $(L\text{-his})_n$ + carboxylic acid (Lindemann and Zundel 1978) systems have already

0340-1057/80/0006/0209/$ 03.00

shown that in $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ hydrogen bonds formed between glutamic acid and histidine residues in proteins in the average largely symmetrical double minimum proton potential wells occur. Thus, these hydrogen bonds are easily polarizable (Janoschek et al. 1972; Zundel 1976) and very large dipole moments (about 10 D) arise (Ratajczak and Sobczyk 1969; Nouwen and Huyskens 1973; Jadżyn and Małeki 1972; Sobczyk 1976) when the equilibrium in these hydrogen bonds is shifted in favour of the polar structure $O^- \cdots H^+N$. It was shown (Lindemann and Zundel 1977a, 1978) that polar environments, i.e., presence of water molecules, shift these proton transfer equilibria in favour of the polar proton limiting structure. Furthermore, it was shown (Lindemann and Zundel 1978) that the degree of proton transfer in these hydrogen bonds, the conformation of $(\text{L-his})_n$, and the degree of hydration are strongly interdependent. On the basis of the large polarizability of these hydrogen bonds, the molecular mechanism of the charge relay system in the active centre of chymotrypsine may be explained (Zundel 1978). This charge relay system was postulated by Blow (1976), to explain the high reactivity of the serine residue responsible for the catalytic effect of this enzyme.

From IR (Zundel and Nagyrevi 1978) as well as from dielectric studies (Ratajczak and Sobczyk 1969; Nouwen and Huyskens 1973; Jadżyn and Małeki 1972; Sobczyk 1976) it is known that $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ bonds in phenol-amine systems may have similar properties. Hence it seems possible that the hydrogen bonds between tyrosine and lysine residues might be easily polarizable proton transfer hydrogen bonds, as well. Such hydrogen bonds could, for instance, occur in the hydrophobic regions of bacteriorhodopsin, as suggested in considering the structure of bacteriorhodopsin proposed by Ovchinnikov (1979), and by Ovchinnikov et al. (1979). Via easily polarizable hydrogen bonds, the protons can be transported by a Grotthus mechanism (Zundel and Weidemann 1971; Zundel 1976, pp 760–761). Easily polarizable proton transfer hydrogen bond systems between side chains are probably the pathways of the protons through hydrophobic regions of biological membranes. Therefore, it seems important to clarify whether tyrosine-lysine bonds are easily polarizable proton transfer hydrogen bonds and can thus take part in such conducting systems.

In the following, using poly-α-amino acids as models the nature of this type of hydrogen bonds between side chains is investigated by IR spectroscopy.

II. Results and Discussion

1. Tyrosine-Lysine Hydrogen Bonds

The nature of the tyrosine-lysine hydrogen bonds was studied with three types of systems: 1. Polymer + monomer systems, i.e., $(\text{L-lys})_n$ with various phenols. 2. The statistical copolymer, copoly $(\text{L-lys, L-tyr})_n$. 3. Mixture of $(\text{L-lys})_n$ + $(\text{L-tyr})_n$. All systems are summarized in Table 1. Figure 1 shows the spectra of selected examples of $(\text{L-lys})_n$ + phenol systems and Figure 2 the spectra of the copoly $(\text{L-lys, L-tyr})_n$ and of the assoziate of $(\text{L-lys})_n$ with high chain length (n = 2286) with $(\text{L-tyr})_n$.

Table 1. Systems and Results

System	Figures	pKa (donor)	ΔpK_a[a]	δOH (cm^{-1})		δOH (cm^{-1}) phenol-phenol for comparison	K_T (dry)	PT %	
				dry	hydrated[b]			dry	hyrated
1	2	3	4	5	6	7	8	9	10
$(L\text{-lys})_n$ + phenol	1a and 3a	9.9[c]	0.5	1245 1230 wsh	1260 1245 sh	1230	0.093	8.5	14
$(L\text{-lys})_n$ + 3-chlorophenol	3b	8.9[c]	1.6	1255	1265 1255 sh	1200	0.482	32.5	46
$(L\text{-lys})_n$ + 3,4-dichlorophenol	3c	8.6[c]	1.9	1290	1300	1220	0.887	47.0	55
$(L\text{-lys})_n$ + 2,4,5-trichlorophenol	1b and 3d	6.7[c]	3.8	–	1290 sh	–	3.34	77.0	84
$(L\text{-lys})_n$ + 2,3,5-trichlorophenol	3e	6.4[c]	4.1	–	–	–	–	100	100
$(L\text{-lys})_n$ + pentachlorophenol	1c and 3f	4.7[c]	5.8	–	–	–	–	100	100
copoly $(L\text{-lys, L-tyr})_n$	2a and 5b	9.8[d]	0.6	1248 1222 sh	1248	dry 1222[e] hyd. 1236	0.17	15	15
$(L\text{-lys})_n$ + $(L\text{-tyr})_n$	2b and 5c	9.8[d]	0.6	1247	1247	dry 1220[f] hyd. 1235	0.17	15	15

[a] pK_a $(L\text{-lys})_n = 10.44$ (Katchalski et al. 1954)
[b] Number of water molecules per acid-base pair, see text
[c] pK_a, from (Drahoňovski and Vacek 1971)
[d] pK_a $(L\text{-tyr})_n = 9.8$ from (Katchalski and Sela 1953)
[e] Hydrobromid
[f] $(L\text{-tyr})_n$

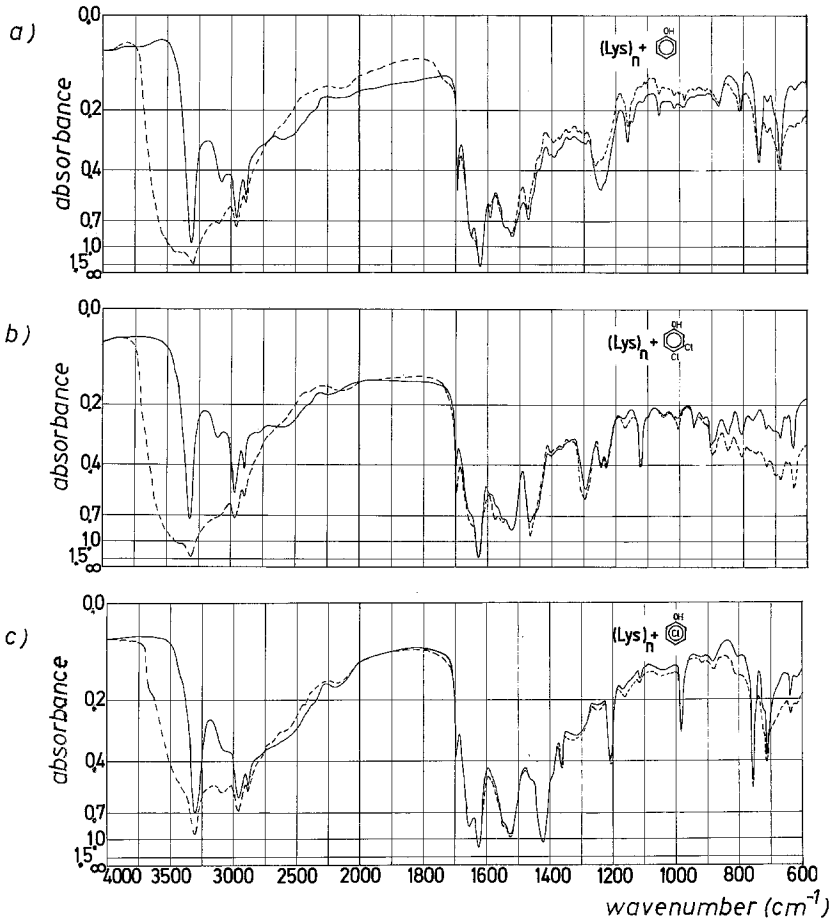

Fig. 1. IR spectra of (L-lys)$_n$ (n = 192) with different chlorophenols: dry film ——; hyrated film at 90% relative humidity — — —. **a** (L-lys)$_n$ + phenol, **b** (L-lys)$_n$ + 3,4 dichlorophenol, **c** (L-lys)$_n$ + pentachlorophenol

1.1 Formation of the Hydrogen Bonds

If non-hydrogen bonded OH groups are present, the stretching vibration of these groups should be observed near 3600 cm^{-1} (Kuhn 1952). In the spectra of all (L-lys)$_n$ + phenol systems (Fig. 1), no such band is found, and in the spectra of the copoly (L-lys, L-tyr)$_n$ and (L-lys)$_n$ + (L-tyr)$_n$ (Fig. 2) only a very weak shoulder is observed at this wave number value. Instead of this band, a very broad band is found in the region 2750–2250 cm^{-1}. This band is caused by the fundamental transitions of the hydrogen bonded groups – as discussed in the following section.

In the spectrum of the hydrobromide of the copoly (L-lys, L-tyr)$_n$ (– – – spectrum in Fig. 2a) a broad band is found in the region 3100–2900 cm^{-1}. This band is caused by the NH^+ stretching vibration in asymmetrical hydrogen bonds

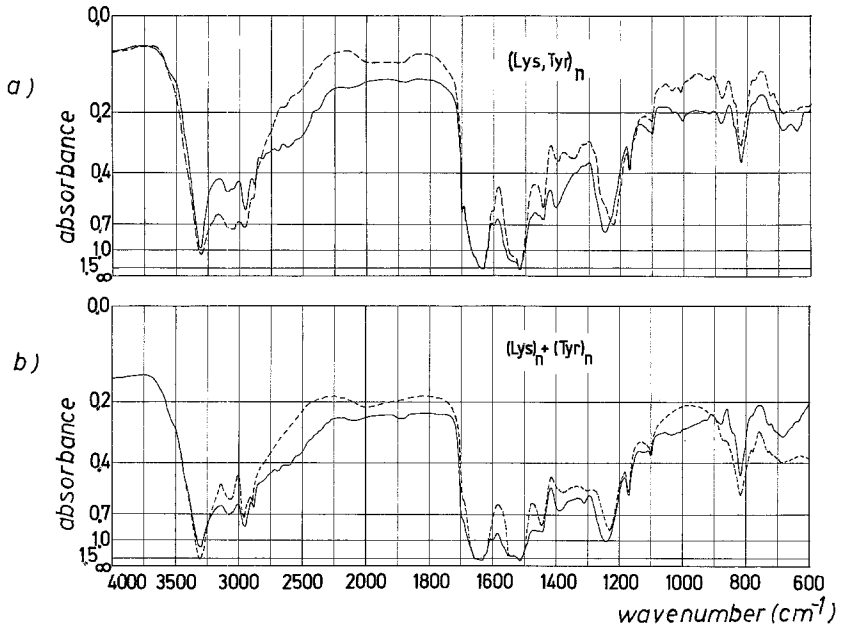

Fig. 2. The IR spectra of **a** copoly (L-lys, L-tyr)$_n$ (n = 110) and **b** (L-lys)$_n$ (n = 2286) + (L-tyr)$_n$: ——. The dashed line in **a** represents the spectrum of copoly (L-lys · HBr, L-tyr)$_n$. The dashed line in **b** represents the sum of the spectra of the isolated partners (L-lys)$_n$ and (L-tyr)$_n$

in amine-hydrohalides (Brisette and Sandorfy 1960). This band disappears with removal of HBr when the OH · · · N $\rightleftharpoons$ O$^-$ · · · H$^+$N bonds are formed which is indicated by the broad band in the region 2750–2250 cm^{-1}. In the spectra of copoly (L-lys, L-tyr)$_n$ and in the spectra of (L-lys)$_n$ + (L-tyr)$_n$, a weak continuous absorption is observed, which begins at the band in the region 2750–2250 cm^{-1} and extends toward smaller wave numbers.

All these results suggest that hydrogen bonds between the phenol OH groups and the lysine residues are formed.

In systems with phenolic OH groups a band of a vibration occurs in the region 1300–1150 cm^{-1} which shows strong OH bending character (Hadži and Bratos 1976). This band is shifted due to hydrogen bond formation, for instance, with the association of phenol from 1179 to 1228 cm^{-1} (Evans 1960). The positions of the respective bands in the systems studied here are given in Table 1, col. 5 (for comparison in col. 7 the position in the pure phenol systems in nujol). With the (L-lys)$_n$ + phenol system, the band at 1245 cm^{-1} indicates nearly quantitative formation of phenol-lysine hydrogen bonds. The very weak shoulder at 1230 cm^{-1} shows that some phenol-phenol associates are present. With the (L-lys)$_n$ + 3-chlorophenol, and with the (L-lys)$_n$ + 3,4-dichlorophenol systems, only one band and no shoulder is found at 1255 or 1290 cm^{-1}, respectively, indicating quantitative formation of phenol-lysine hydrogen bonds. The shift of this band (1245, 1255, 1290 cm^{-1}) in this series of systems demonstrates that the phenol-lysine hydrogen bonds become increasingly

stronger in this series of systems corresponding to the increasing acidity of the phenols. With the other (L-lys)$_n$ + phenol systems, this band is weak or no longer observed because – as discussed in the next section – the proton transfer equilibrium OH $\cdots$ N $\rightleftharpoons$ O$^-$ $\cdots$ H$^+$N shifts more and more towards the polar structure.

In the case of the copoly (L-lys, L-tyr)$_n$, with the removal of HBr the respective band shifts from 1222 to 1248 cm^{-1}, indicating the formation of hydrogen bonds between the tyrosine and lysine residues (Fig. 2a). Only a very weak shoulder remains at 1222 cm^{-1}, indicating that some tyrosine groups are associated with one another. A quantitative estimation shows that the amount of these groups is less than 10%.

In the case of the (L-lys)$_n$ + (L-tyr)$_n$ system, this band is also found at 1248 cm^{-1}. The comparison with the pure (L-tyr)$_n$ system, where the band occurs at 1222 cm^{-1}, indicates hydrogen bond formation between the tyrosine and lysine residues (Fig. 2b). The complete disappearance of the band at 1222 cm^{-1} indicates that no self association of tyrosine groups occurs.

In summary we can state: In all water-free systems the lysine residues and phenol groups are almost quantitatively associated via OH $\cdots$ N $\rightleftharpoons$ O$^-$ $\cdots$ H$^+$N hydrogen bonds. Only in the copoly (L-lys, L-tyr)$_n$ and in the (L-lys)$_n$ + (L-tyr)$_n$ systems few non-hydrogen bonded phenol OH groups are present. Furthermore, in the (L-lys)$_n$ + phenol and in the copoly (L-lys, L-tyr)$_n$ systems, some self-associated phenol or tyrosine residues are found, but their number is less than 10%.

1.2. The Position of the Proton Transfer Equilibrium

Let us consider first the (L-lys)$_n$ + phenol systems. The position of the proton transfer equilibrium can be determined from bands which occur only in the spectrum of phenol or of the phenolate. The bands used for the determination of the degree of proton transfer are shown in Figure 3 for the water-free and the hydrated samples. The evaluation of these bands is described in the experimental section. The results are given in Table 1, cols. 8–10. In Figure 4a, the percentage of proton transfer is plotted against the ΔpK$_a$ (pK$_a$ of the protonated N-base minus pK$_a$ of the phenol). In Figure 4b the data are plotted according to Huyskens and Zeegers-Huyskens (1964). Figure 4a shows that the two proton limiting structures OH $\cdots$ N $\rightleftharpoons$ O$^-$ $\cdots$ H$^+$N have equal weights in a system with ΔpK$_a$ = 2.2. The same result is obtained from the Huyskens plot in Figure 4b. The system having mostly symmetrical hydrogen bonds is the (L-lys)$_n$ + 3,4-dichlorophenol combination. Double minimum proton potential wells should be present in the transfer region. If considered isolated, these hydrogen bonds are easily polarizable (Janoschek et al. 1972). In the real system they are more or less strongly polarized (Pfeiffer et al. 1979). The deeper well of the proton potential in the (L-lys)$_n$ + phenol system is preferentially at the phenol, and in the higher substituted chlorophenols preferentially at the lysine residue.

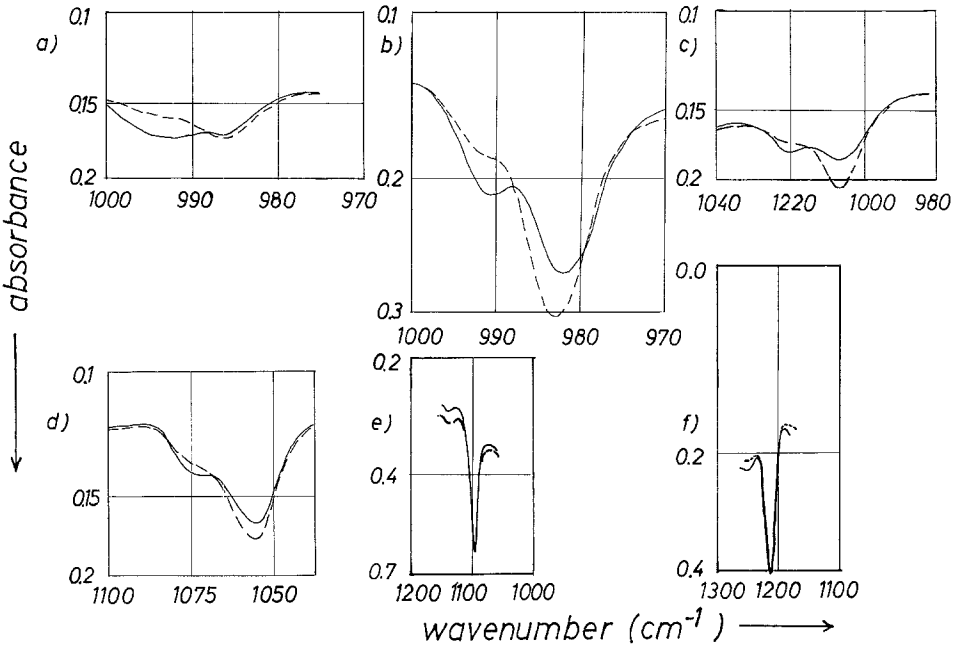

Fig. 3. Determination of the proton transfer in the complexes of (L-lys)$_n$ with different chlorophenols by means of characteristic bands of the chlorophenols. Dry film: ——; hydrated film at 90% relative humidity: — — —; **a** phenol; **b** 3-chlorophenol; **c** 3,4-dichlorophenol; **d** 2,4,5-trichlorophenol; **e** 2,3,5-trichlorophenol; f pentachlorophenol

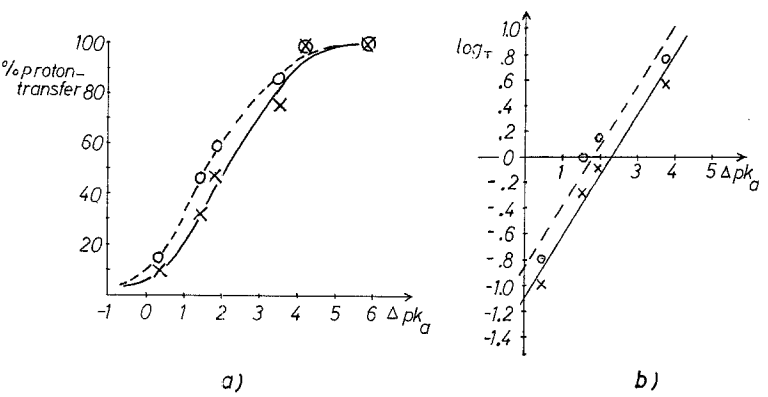

Fig. 4. (L-lys)$_n$ + chlorophenol systems. Dry systems: ——; hydrated systems: — — —; **a** degree of the proton transfer plotted over ΔpK_a; **b** logarithmic plot of the transfer-constant K_T plotted over ΔpK_a

The copoly (L-lys, L-tyr)$_n$ and the (L-lys)$_n$ + (L-tyr)$_n$ systems: Figure 5a shows a ring vibration found with (L-tyr)$_n$ at 1513 cm^{-1} and with (L-tyr$^-$Na$^+$)$_n$ at 1497 cm^{-1}. From these bands the position of the transfer equilibria can be determined. Figure 5b shows spectra of the HBr containing (– – – line), and HBr free species (—— line). Furthermore, in Figure 5c the spectrum of

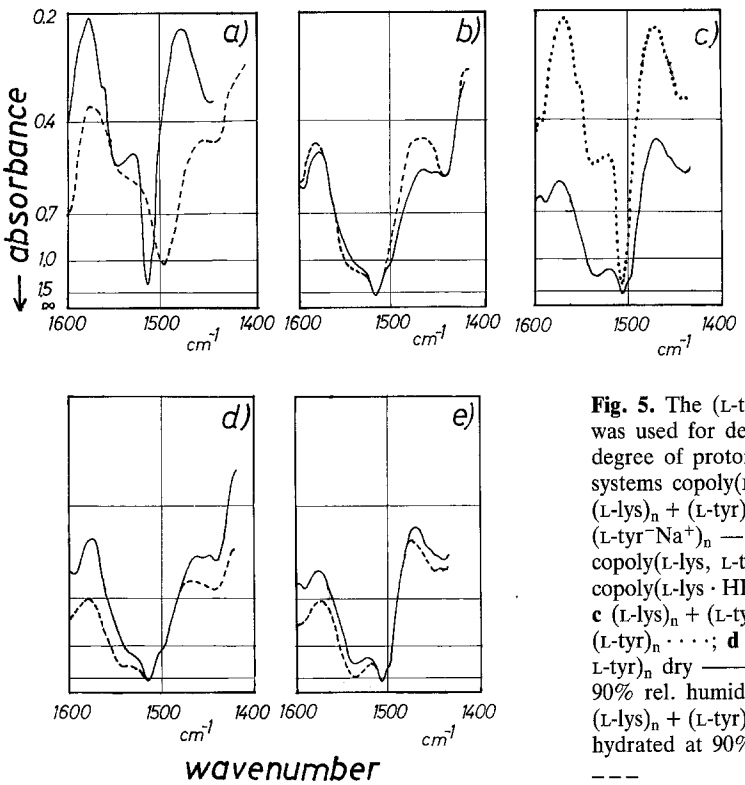

Fig. 5. The $(\text{L-tyr})_n$-band, which was used for determining the degree of proton transfer in the systems copoly(L-lys, L-tyr)$_n$ and $(\text{L-lys})_n + (\text{L-tyr})_n$. **a** $(\text{L-tyr})_n$ ——, $(\text{L-tyr}^-\text{Na}^+)_n$ ———; **b** copoly(L-lys, L-tyr)$_n$ ——; copoly(L-lys · HBr, L-tyr)$_n$ – – –; **c** $(\text{L-lys})_n + (\text{L-tyr})_n$ ——, $(\text{L-tyr})_n$ · · · ·; **d** copoly(L-lys, L-tyr)$_n$ dry ——; hydrated at 90% rel. humidity ———; **e** $(\text{L-lys})_n + (\text{L-tyr})_n$ dry ——; hydrated at 90% rel. humidity – – –

$(\text{L-lys})_n + (\text{L-tyr})_n$ is shown (—— line), and for comparison the spectrum of $(\text{L-tyr})_n$ (· · · line).

In the HBr containing copoly (L-lys, L-tyr)$_n$, only the $(\text{L-tyr})_n$ band at 1513 cm^{-1} is observed. With the HBr free copoly (L-lys, L-tyr)$_n$, and with $(\text{L-lys})_n + (\text{L-tyr})_n$, a pronounced shoulder at the low wave number slope of this band (at about 1500 cm^{-1}) occurs, demonstrating the presence of tyrosine residues from which the proton has been removed. Thus, the polar proton limiting structure in the $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ bonds formed between the tyrosine and lysine residues has noticeable weight. A quantitative evaluation shows that 10–20% transfer of the protons to the lysine residues occurs.

Thus, hydrogen bonds formed between tyrosine and lysine residues are proton transfer hydrogen bonds which are represented by the two proton limiting structures $OH \cdots N \rightleftharpoons O^- \cdots H^+N$.

Phenol-amine hydrogen bonds are relatively long (2.7–2.9 Å) (Vinogradov 1979). A comparison with calculated line spectra [Fig. 6c in (Janoschek et al. 1973)] and with the calculated spectra for long hydrogen bonds with double minimum [Fig. 5c in (Hayd et al. 1979)] shows that the broad band observed in the region 2750–2250 cm^{-1} is caused by the fundamental transitions in these hydrogen bonds. This is in good agreement with the calculated spectra of long, easily polarizable hydrogen bonds and with experimental results from monomer

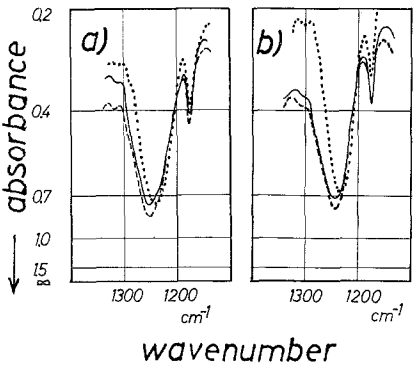

Fig. 6. Vibration of the tyrosine-residues with δ_{OH} character as indicator for the formation of hydrogen bonds between tyr – and lys – residues. Dry film: ——; hydrated film at 90% rel. humidity — — —; **a** copoly (L-lys, L-tyr)$_n$; hydrated copoly (L-lys · HBr, L-tyr)$_n$ · · · ·; **b** (L-lys)$_n$ + (L-tyr)$_n$; hydrated (L-tyr)$_n$ at 90% rel. humidity for comparison: · · · ·

-monomer phenol-amine systems (Zundel and Nagyrevi 1978). From this band a less intense continuous absorption extends toward smaller wave numbers (Fig. 2). This continuous absorption confirms that tyrosine-lysine hydrogen bonds are easily polarizable ones.

1.3. Changes with Hydration

In Figure 1 the dashed spectra were recorded from samples hydrated in air of 90% relative humidity. The OH stretching vibration of the adsorbed water molecules is observed as a broad band in the region 3700–3200 cm^{-1}. As determined with a pure (L-lys)$_n$ film at 90% relative air humidity, 3.1 molecules of water were attached per lysine residue. This value is 25% less than the value given by Breuer and Kennerly (1971) but in good agreement with the hydration number of 3.0 per lysine residue in serum albumin determined by Oakes (1976).

Comparing the gravimetrical with the spectroscopic data we calibrated the OH stretching vibration. On the basis of this result with the (L-lys)$_n$ + phenol systems, the number of attached water molecules can be estimated roughly. In the films with hydrophilic phenols (phenol, 3-chlorophenol, 3,4-dichlorophenol), about 3 water molecules are present per lysine residue, whereas with the more hydrophobic phenols (2,3,5- and 2,4,5-trichlorophenols, pentachlorophenol), only 1–2 molecules of water are present per lysine residue.

The results plotted in Figure 4 demonstrate that the addition of water molecules shifts the proton transfer curve a little toward smaller ΔpK_a values (ΔpK_a at which the system is symmetrical on an average, hydrated 1.8, water-free 2.2). Thus, the water molecules shift the proton transfer equilibrium in favour of the polar proton limiting structure, an effect already well known from other types of proton transfer systems (Lindemann and Zundel 1977a, 1978; Jadżyn and Małeki 1972; Schreiber et al. 1978; Pawlak and Magoński 1980).

As shown in the following, increasing degree of hydration also favours the disassociation of the phenol-lysine hydrogen bonds. This is indicated by the observation that the broad band in the region 2750–2250 cm^{-1} – assigned to the

fundamental transitions in the phenol-lysine hydrogen bonds – disappears increasingly with increasing degree of hydration (Fig. 1, comparison of the —— and – – – spectra). This disassociation effect can easily be studied by observing the band of the δOH vibration (cf. in Table 1, cols. 5 and 6). This band shifts with the (L-lys)$_n$ + phenol system from 1245 to 1260 cm^{-1}, with the (L-lys)$_n$ + 3-chlorophenol system from 1255 to 1265 cm^{-1} and with the (L-lys)$_n$ + 3,4-chlorophenol system from 1290 to 1300 cm^{-1}, i.e., the position in which this band is observed in pure diluted solutions of the phenols in water. Thus, this shift of the band proves that the phenol-lysine hydrogen bonds are broken, and phenol-water bonds are formed. With all these systems a weak shoulder remains at the position where this band is observed, as long as the phenol-lysine bonds are formed, demonstrating that not all these bonds are broken by the water molecules. With the other (L-lys)$_n$ + phenol systems, the disassociation of these hydrogen bonds cannot be studied by observing this band, since, as already mentioned, due to increasing proton transfer, the δOH vibration no longer occurs. Vinogradov (1970) has shown, however, that increasing weight of the polar structure in the $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ equilibrium favours the disassociation of the hydrogen bonds. Hence, the disassociation of the hydrogen bonds should take place in these systems to a larger degree than in the system with the less acidic phenols. Figure 1c shows, however, that with the (L-lys)$_n$ + pentachlorophenol systems, the band in the region 2750–2250 cm^{-1} decreases less strongly with hydration, although in this system 100% proton transfer occurs. This observation, however, is not in disagreement with Vinogradov's results, since the degree of hydration of the films with the hydrophobic phenols is much less – as already mentioned.

The copoly (L-lys, L-tyr)$_n$ and the (L-lys)$_n$ + (L-tyr)$_n$ systems: A rough estimation, from the OH stretching vibration of the water molecules, shows that 1–2 water molecules per lysine residue are adsorbed at 90% air humidity.

These water molecules do not shift the proton transfer equilibrium, since neither the band at 1514 cm^{-1} nor the shoulder at 1500 cm^{-1} changes in intensity with hydration (see Figs. 5d and e). Furthermore, no change of the band at 1247 cm^{-1} is observed with hydration (cf. —— and — — — spectra in Figs. 6a and b). Thus, the $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ hydrogen bonds are not broken when 1–2 water molecules are added.

In summary we can state: At 90% rel. humidity, in the (L-lys)$_n$ + phenol systems with hydrophilic phenols, about 3 water molecules per lysine residue are added, and in systems with hydrophobic phenols and with the copoly (L-lys, L-tyr)$_n$ and the (L-lys)$_n$ + (L-tyr)$_n$ systems, only 1–2. In the case of the (L-lys)$_n$ + phenol systems, the proton transfer curve is shifted a little toward smaller ΔpK_a values, i.e., in favour of proton transfer. In the systems in which 3 water molecules per lysine residue are attached, the phenol-lysine hydrogen bonds are broken to a large extent, only few bonds remain. With the copoly (L-lys, L-tyr)$_n$ and the (L-lys)$_n$ + (L-tyr) systems, the number of 1–2 water molecules per lysine residue is not enough to shift the equilibrium or to break the tyrosine-lysine hydrogen bonds.

Table 2. IR Bands and Conformations

Substance	Figure	Amide A (cm^{-1})	Amide I (cm^{-})	Amide II (cm^{-1})	Conformation
$(L\text{-lys})_n$ (n = 2286)	7	3292 s	1544 s	1650 s	α-helix
$(L\text{-lys})_n$ (n = 192	7	3271 s	1623 s 1692 sh 1650 sh	1535 s	Antiparallel β-structure + few α-helix
$(L\text{-lys})_n$ + phenol	1a	3274	1623 s 1649 sh 1691 m	1530 s 1540 sh	Antiparallel β-structure + few α-helix
$(L\text{-lys})_n$ + 3-chlorophenol	–	3273 s	1623 s 1650 sh 1692 m	1545 s	Antiparallel β-structure + few α-helix
$(L\text{-lys})_n$ + 3,4-dichlorophenol	1b	3272 s	1624 s 1650 sh 1692 m	1540 sh 1528 m	Antiparallel β-strukture + few α-helix
$(L\text{-lys})_n$ + 2,4,5-trichlorophenol	–	3276 s	1623 s 1650 s 1692 w	1540 s	Antiparallel β-structure + α-helix
$(L\text{-lys})_n$ + 2,3,5-trichlorophenol	–	3284	1650 s 1625 sh	1542 s	α-helix + few antiparallel β-structure
$(L\text{-lys})_n$ + pentachlorophenol	1c	3275 s	1651 s 1622 s	1543 (sh) 1530 s	Antiparallel β-structure α-helix
Copoly $(L\text{-lys} \cdot HBr, L\text{-tyr})_n$	2a	3175 s	1625 s 1652 sh 1695 sh	1530–35 s	Antiparallel β-structure + few coil
Copoly $(L\text{-lys}, L\text{-tyr})_n$	2a	3279 s	1625 s 1652 sh 1695 sh	1530–35 s	Antiparallel β-structure + few coil
$(L\text{-lys})_n$ + $(L\text{-tyr})_n$	2b	3288 s	1650 s 1630 s 1690 sh	1540 s	Mixture of α-helix and antiparallel β-structure
$(L\text{-tyr})_n$	–	3279 s	1629 s 1690 sh	1535–1540 s	Antiparallel α-structure

1.4 Conformation of the Peptide Backbone

The conformation of the peptide backbone can be determined from the amide bands (Elliot 1954; Miyazawa and Blout 1961; Katchalski et al. 1964; Schellman and Schellman 1974; Chirgadže et al. 1976). These bands are summarized in Table 2.

Free polybase $(L\text{-lys})_n$: Conformational changes dependent on the degree of hydration – as observed in films of $(L\text{-lys} \cdot HCl)_n$ and $(L\text{-lys} \cdot HBr)_n$ (Chirgadže

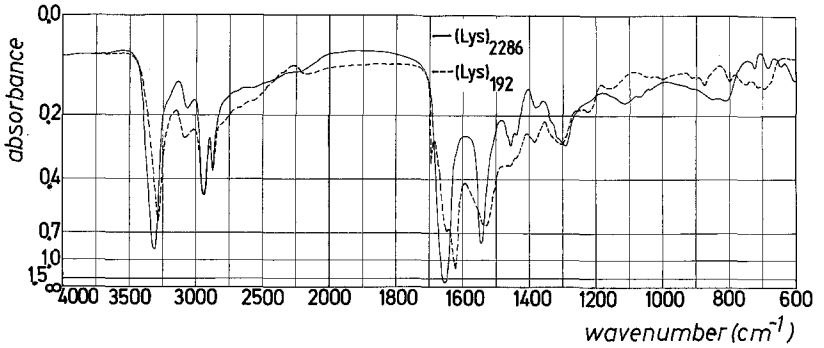

Fig. 7. The IR spectrum of (L-lys)$_n$ with different chain lengths. n = 2286: ——; n = 192: — — —

and Ovsepyan 1972; Ikeda et al. 1974) – are not observed with the free polybase (L-lys)$_n$.

The conformation is, however, dependent on the chain length, as shown in Figure 7. The amide bands (see Table 2) demonstrate that (L-lys)$_{2286}$ is present α-helical, whereas (L-lys)$_{192}$ is present in antiparallel β-structure. Immediately after the preparation of the films by drying, also the (L-lys)$_{192}$, films are present in α-helical conformation, but if they are hydrated within a few days at 90% rel. air humidity, they irreversibly change to antiparallel β-structure. With (L-lys)$_{2286}$, such a conformational change can not be induced.

(L-lys)$_n$ with n = 192 + phenols: A consideration of the amide bands in Figure 1 and Table 2 shows that (L-lys)$_n$ is more or less α-helical or in β-structure in the various samples. With hydration over a longer period of time, however, (L-lys)$_n$ in all (L-lys) + phenol systems also shows a tendency to change to β-structure. There is no systematic correlation between the conformation of (L-lys)$_n$ and the transfer of the proton in the hydrogen bonds formed between the side chains – as observed with (L-his)$_n$ + carboxylic acid systems (Lindemann and Zundel, 1978) and with (L-cys)$_n$ + N-base systems (Kristof and Zundel).

(L-lys)$_n$ + (L-tyr)$_n$: A consideration of the amide I band in Figure 8 and Table 2 shows that in the films of the pure polymers, (L-lys)$_n$ is present α-helical, and (L-tyr)$_n$ in β-structure. In the mixture the same amide bands are observed. Thus both polymer molecules have not changed their conformation. Despite of this fact, as already demonstrated in Sect. II. 1.1., the hydrogen bonds between the side chains can be formed nearly quantitatively.

In summary we can state that in all three types of systems, neither the formation of $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ hydrogen bonds between lysine residues and phenols, or between the side chains of lysine and tyrosine residues, nor the proton transfer in these bonds induces a conformational change of the peptide backbone. In this regard, the behaviour of the systems studied in this paper is different, compared with (L-his)$_n$ + carboxylic acid (Lindemann and Zundel 1978) and with (L-cys)$_n$ + N-base (Kristof and Zundel) systems.

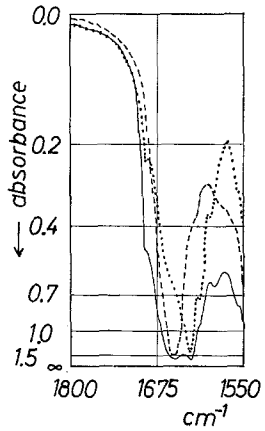

Fig. 8. The amide I band of (L-lys)$_n$ + (L-tyr)$_n$: ——; for comparison the amide I band of (L-lys)$_n$ (n = 2286): — — —, and the amide I band of (L-tyr)$_n$: · · ·

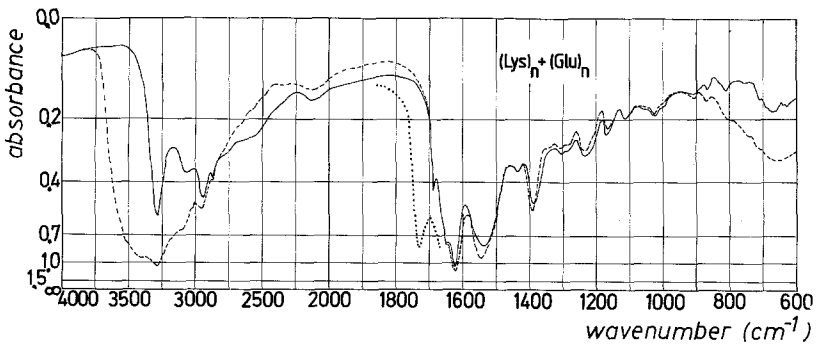

Fig. 9. IR-spectra of (L-glu)$_n$ + (L-lys)$_n$. Dry film: ——; hydrated film at 90% relative humidity: — — —; $\nu_{C=0}$ band of (L-glu)$_n$: · · · ·

2. *Glutamic Acid – Lysine Hydrogen Bonds*

Figure 9 shows spectra of a (L-lys)$_n$ + (L-glu)$_n$ film. Proton transfer equilibria in hydrogen bonds, in which carboxylic acid groups are involved, can easily be determined from $\nu C = 0$ of the -COOH groups, or from ν_{as} and ν_s of the $-CO_2^-$ ions (Lindemann and Zundel 1977a, 1977b, 1978). For comparison, the $\nu C = 0$ band of (L-glu)$_n$ observed at 1715 cm^{-1} is shown in Figure 9 as a dotted line. This band is not observed in the spectra of (L-lys)$_n$ + (L-glu)$_n$. Instead of this band, ν_s of the anionic group $-CO_2^-$ is found at 1395 cm^{-1}. Thus all protons are present at the lysine residues as would be expected from the pK_a values of the polylysine [pK_a = 10.44 (Katchalski et al. 1954)] and of the polyglutamic acid [pK_a = 4.45 (Nagasawa and Holtzer 1964)].

Thus, $O^- \cdots H^+N$ bonds with asymmetrical single minimum proton potentials occur in the hydrogen bonds between glutamic acid and lysine residues.

Results with similar hydrogen bonds show (Ratajczak and Sobczyk 1969; Nouwen and Huyskens 1973; Jadżyn and Małeki 1972; Sobczyk 1976) that the dipole moments of such hydrogen bonds are about 10 D, i.e., very large.

In the water-free system (—— line), the formation of these hydrogen bonds is indicated by the broad band in the region 2750–2250 cm^{-1}. This band vanishes completely when water molecules are added (— — — line). A rough estimation of the water content of this sample shows that 3–4 water molecules are present per lysine residue. Thus, these polar hydrogen bonds are easily broken, even by the presence of few water molecules, a result which is in good agreement with electrochemical findings obtained by Vinogradov (1970).

The amide bands (amide A at 3271 cm^{-1}, amide II at 1626 cm^{-1} with a weak shoulder at 1692 cm^{-1}) show that the backbones of (L-glu)$_n$ and (L-lys)$_n$ are largely present in antiparallel β-structure. The weak shoulder at 1651 cm^{-1} shows that coiled or α-helical regions are also present, but these regions are very small.

III. Conclusions

In water-free (L-lys)$_n$ + phenol systems, $OH \cdots N \rightleftharpoons O^- \cdots H^+N$ hydrogen bonds are formed. They are symmetrical on an average if the difference between the pK_a of the protonated lysine residue and the phenol is 2.2. Regarding the pK_a value of protonated lysine and tyrosine residues, hydrogen bonds between these groups should be easily polarizable with a double minimum proton potential. This is confirmed by studies of copoly (L-lys, L-tyr)$_n$ and (L-lys)$_n$ + (L-tyr)$_n$. In these systems, the weights of the proton limiting structure $OH \cdots N$ is 80–90%, and that of the $O^- \cdots H^+N$ structure, 10–20%, i.e., the proton is preferentially present at the tyrosine residue. In contrast to this result, in the $O^- \cdots H^+N$ hydrogen bonds between the glutamic acid and lysine residues, single minimum proton potentials with the well at the lysine residues are present, i.e., these bonds are highly asymmetrical and the dipole moment of these hydrogen bonds is very large (about 10 D).

In the phenol-lysine hydrogen bonds, hydration shifts the proton transfer equilibrium a little in favour of the polar $O^- \cdots H^+N$ proton limiting structure. These hydrogen bonds, however, are already broken to a large extent when only about three water molecules are present per lysine residue. If these bonds are present in hydrophobic regions, however, they remain quantitatively formed, as shown by the copoly (L-lys, L-tyr)$_n$ and by the (L-lys)$_n$ + (L-tyr)$_n$ systems.

This situation, for instance, occurs if such groups are present in hydrophobic regions of membrane proteins. The structure proposed for bacteriorhodopsin by Ovchinnikov et al. (1979) suggests that such bonds can be formed between side chains of this protein. Thus, with respect to their proton transfer properties hydrogen bonds between tyrosine and lysine residues may participate in the proton conducting, hydrogen bonded systems performing the proton transport through hydrophobic regions of biological membranes.

IV. Material and Methods

Preparation of the Free Polybase (L-lys)$_n$

(L-lys)$_n$ was purchased as hydrochloride from Miles Laboratories with mean chain lengths n = 192 and n = 2286. For preparing the free polybase, 10 ml of an aqueous solution of (L-lys · HCl)$_n$ with a concentration of 30–35 μ equivalents/ml were passed through a column filled with a strong basic anion exchanging resin (Merck, Darmstadt, Lewatit MP 5080). The Cl^- free solutions of the free polybase obtained in this way had a pH value of 11.1. The concentration of basic ε-amino groups in these solutions was determined by potentiometric titration. Values between 18 and 25 μ equivalents/ml were obtained. To exclude absorption of CO_2, freshly distilled water was used and all procedures were performed in a carbondioxide-free glove box or under a nitrogen atmosphere. The pK_a value of the (L-lys)$_n$ obtained by this method was determined to 10.5 ± 0.1 by a titration at constant ionic strength (Katchalski et al. 1954). This value is in good agreement with the value obtained by Katchalski et al. (1954).

To be sure that no racemization or cleavage of the backbone under the alkaline conditions in the exchange resin had occured, the value of the optical rotation $[\alpha]_D$ was determined before and after the treatment with the exchanging resin. In both cases, a value of $[\alpha]_D = -84° \pm 1°$ at pH = 5.5 was obtained, indicating that no racemization and no cleavage of the backbone occurred.

Preparation of the Films

The films of the investigated substances were obtained from aqueous solutions by a centrifugation drying procedure (Hofmann and Zundel 1971)

Films of (L-lys)$_n$

The films were obtained by drying the aqueous solutions of the free polybase described above.

Films of (L-lys)$_n$ with chlorophenols

To obtain a 1 : 1 mixture, films of 10 μ equivalents of (L-lys) with n = 192 were put in a desiccator together with the phenol. To achieve a higher rate of absorption the phenols were heated and the germanium supports with the (L-lys)$_n$ films were cooled. The amount of absorbed phenol could be determined by the intensity of the phenol UV-absorption after dissolving the film in a 1 : 1 mixture of ethanol and aqueous 0.01 n HCl.

Films of copoly (L-lys, L-tyr)$_n$

Copoly (L-lys · HBr, L-tyr)$_n$ with the mean chain length n = 110 was purchased from Miles Laboratories. The corresponding films could be prepared from

aqueous solutions. To remove the HBr, the films were carefully treated several times with 0.01 n NaOH until the supernatant NaOH solution was free of Br^-. Thereafter, remaining NaOH was removed by washing with freshly distilled water. All procedures were performed under a nitrogen atmosphere.

Films of (L-lys)$_n$ with n = 2286 + (L-Tyr)$_n$

These films were obtained from aqueous solutions of the two components in a 1 : 1 ratio. To achieve a sufficient solubility, a few drops of concentrated ammonia were added. The mean chain length of (L-*tyr*)$_n$ was n = 24.

Films of (L-lys)$_n$ + (L-glu)$_n$

These films were prepared from aqueous solutions in a manner similar to the (L-lys)$_n$ + (L-tyr)$_n$ films. To avoid precipitation, some drops of pyridine, instead of ammonia, were added until the solutions became nearly clear. During centrifugation, the pyridine evaporated and the obtained films were free of pyridine.

IR Measurements

The measurements were performed using special IR cells which were described elsewhere (Zundel 1969). These cells could be evacuated. Definite hydration of the sample was achieved via definite vapour preassures over saturated salt solutions.

A Perkin-Elmer 325 IR-gratingspectrophotometer was used for all measurements (slit program 8, time response 2).

Determination of the proton transfer in the (L-lys)$_n$ + phenol systems:

As reference systems for determining the degree of the proton transfer, characteristic bands of the chlorophenol or of the corresponding sodium salt, respectively, were used. For calibrating, definite mixtures of chlorophenol and the corresponding sodium salt were suspended in nujol and the ratios of the intensities of the characteristic bands were plotted against the molar ratios of the two components in the suspension. Using this calibration curve it was possible to determine the weights of the proton limiting structures of the proton transfer equilibria, evaluating the intensity ratio of the characteristic bands in the (L-lys)$_n$ + chlorophenol spectra.

Acknowledgement. Our thanks are due to the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for providing the facilities for this work.

References

Blow DM (1976) Acc Chem Res 9: 145–152
Breuer MM, Kennerly MG (1971) J Colloid Interface Sci 37: 124–131
Brisette C, Sandorfy C (1960) Can J Chem 38: 34–44

Chirgadže YuN, Ovsepyan AM (1972) Biopolymers 11: 2179–2186
Chirgadže YuN, Brazhnikov EV, Nevskaya NA (1976) J Mol Biol 102: 781–792
Christensen JJ, Hansen LD, Izatt RM (eds) (1976) Handbook of proton ionisation heats. J. Wiley, New York
Drahonovski J, Vacek Z (1971) Collect Czech Chem Comm 36: 3431–3440
Elliot A (1954) Proc R Soc A221: 104–114
Evans JC (1960) Spectrochim Acta 16: 1382–1392
Hadži D, Bratos S (1976) In: Schuster P, Zundel G, Sandorfy C (eds) The hydrogen bond – Recent developments in theory and experiments, vol. II. North Holland, Amsterdam
Hayd A, Weidemann EG, Zundel G (1979) J Chem Phys 70: 86–91
Hofmann KP, Zundel G (1971) Rev Sci Instrum 42: 1726–1727
Huyskens P, Zeegers-Huyskens Th (1964) J Chim Phys 61: 81–86
Ikeda S, Kito A, Imae T (1974) J Colloid Interface Sci 48: 256–262
Jadżyn J, Małeki J (1972) Acta Phys Pol A41: 599–616
Janoschek R, Weidemann EG, Pfeiffer H, Zundel G (1972) J Amer Chem Soc 94: 2387–2396
Janoschek R, Weidemann EG, Zundel G (1973) J Chem Soc, Faraday Trans 2 69: 505–520
Katchalski E, Sela M (1953) J Am Chem Soc 75: 5284–5289
Katchalski E, Shavit N, Eisenberg H (1954) J Polymer Sci 13: 69–84
Katchalski E, Sela M, Silmann HI, Berger A (1964) In: Neurath H (ed) The proteins, vol. II. Academic Press, New York, pp 405–602
Kristof W, Zundel G (in preparation)
Kuhn J (1952) J Am Chem Soc 74: 2492–2499
Lindemann R, Zundel G (1977a) Biopolymers 16: 2407–2418
Lindemann R, Zundel G (1977b) J Chem Soc, Faraday Trans 2 73: 788–803
Lindemann R, Zundel G (1978) Biopolymers 17: 1285–1304
Miyazawa T, Blout ER (1961) J Am Chem Soc 83: 712–719
Murray J, Gordon N (1935) J Am Chem Soc 57: 110–111
Nagasawa M, Holtzer A (1964) J Am Chem Soc 86: 538–543
Nouwen R, Huyskens P (1973) J Mol Struct 16: 459–471
Oakes J (1976) J Chem Soc, Faraday Trans 1 72: 216–227
Ovchinnikov YuA (1979) Eur J Biochem 94: 321–336
Ovchinnikov YuA, Abdulaev NG, Feigina MJu, Kiselev AV, Lobanov NA (1979) FEBS Lett 100: 219–224
Pawlak Z, Magoński J (1980) J Solution Chem
Pfeiffer H, Zundel G, Weidemann EG (1979) J Phys Chem 83: 2544–2551
Ratajczak H, Sobczyk L (1969) J Chem Phys 50: 556–557
Schellman J, Schellman C (1974) In: Neurath H (ed) The proteins, vol. II. Academic Press, New York, pp 1–137
Schreiber M, Koll A, Sobczyk L (1978) Bull Acad Pol Sci Ser Sci Chim 26: 651–654
Sobczyk L (1976) In: Schuster P, Zundel G, Sandorfy C (eds) The hydrogen bond – Recent developments in theory and experiments, vol III. North Holland, Amsterdam, pp 936–963
Vinogradov SN (1970) Biochim Biophys Acta 214: 6–27
Vinogradov SN (1979) Biopolymers 18: 1559–1561
Weast RC (1977/78) Handbook of chemistry and physics. 58th ed. CRC-Press, West Palm Beach
Zundel G (1969) Hydration and intermolecular interaction. Academic Press, New York (1972: Mir. Moscov)
Zundel G (1976) In: Schuster P, Zundel G, Sandorfy C (eds) The hydrogen bond – Recent developments in theory and experiments, vol II. North Holland, Amsterdam, pp 683–766
Zundel G (1978) J Mol Struct 45: 55–73
Zundel G, Nagyrevi A (1978) J Phys Chem 82: 685–689
Zundel G, Weidemann EG (1971) In: Broda E, Locker A, Springer-Lederer H (eds) First European Biophysics Congress, vol 6. Wiener Medizinische Akademie, Wien, pp 43–47

Received December 27, 1979/Accepted March 25, 1980