GLUTAMIC ACID–DIHYDROGEN PHOSPHATE HYDROGEN-BONDED NETWORKS: THEIR PROTON POLARIZABILITY AS A FUNCTION OF CATIONS PRESENT

Infrared Investigations

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ABSTRACT Glutamic acid [$(\text{L-glu})_n$] + dihydrogen phosphate systems are studied by infrared (IR) spectroscopy dried and hydrated at 75% relative humidity, as a function of both the phosphate–glutamic acid residue (P_i/glu) ratio and the type of cations present.

It is shown that the glutamic acid groups form hydrogen-bonded chains with the phosphates. In these chains the positive charge fluctuates, and they show very large proton polarizability which increases in the series Li^+, Na^+, K^+ systems. These chains are cross-linked via phosphate–phosphate hydrogen bonds, in which the proton is almost localized at one P_i.

The comparison of the $(\text{L-glu})_n$ + dihydrogen phosphate systems with the results obtained earlier in the case of $(\text{L-glu})_n$ + hydrogen phosphate systems shows that the behavior of $(\text{L-glu})_n + P_i$ systems strongly depends on the pH. Only with decreasing pH the conducting chains are formed.

Finally, a hypothesis is discussed with regard to the charge conduction in the F_0 subunit of the H^+-ATPase in mitochondria.

INTRODUCTION

In almost all proteins glutamic and aspartic acid groups are present. Furthermore, in many biological systems phosphates are decisively important for the conformation of proteins, e.g., actin (1), and for the functions of systems, e.g., the conversion of chemical into mechanical energy (2).

It is of particular interest that phosphates have a pK_a value in the physiological pH region (3). Hence, with increasing pH a hydrogen bond donor group becomes an acceptor or with decreasing pH an acceptor becomes a hydrogen bond donor group. Thus, the behavior of such systems should change considerably as a function of the pH.

In preceding papers we studied $(\text{L-lys})_n$ + dihydrogen phosphate (4) and $(\text{L-his})_n$ + hydrogen phosphate systems (5). In both cases the side chain phosphate hydrogen bonds are proton transfer hydrogen bonds with large proton polarizability (6, 7), as indicated by intense infrared (IR) continua (8, 9). In some of these systems the residues form with several phosphate (P_i) hydrogen-bonded chains having very large proton polarizability, which are suitable for charge conduction.

In reference 10 glutamic acid [$(\text{L-glu})_n$] + hydrogen phosphate systems have been studied. In all systems $\text{COH}\cdots{}^-\text{OP} \rightleftharpoons \text{CO}^-\cdots\text{HOP}$ hydrogen bonds with large proton polarizability are formed, whereby the position of the proton transfer equilibria in these bonds depends very sensitively on the type of cations present and on the degree of hydration. It is, however, of particular interest that in these systems no conducting hydrogen-bonded chains are formed by one residue with several hydrogen phosphates.

Here $(\text{L-glu})_n$ + dihydrogen phosphate systems are studied and compared with the behavior of the $(\text{L-glu})_n$ + hydrogen phosphate systems.

METHOD

$(\text{L-glu})_n$ was purchased from Miles GmbH (München, FRG) with a mean chain length of $n = 275$. $NaH_2PO_4 \cdot H_2O$, KH_2PO_4, and H_3PO_4 were purchased from E. Merck (Darmstadt, FRG) and Fluka (Neu-Ulm, FRG), respectively. The LiH_2PO_4 solution was obtained by a potentiometric titration of an aqueous H_3PO_4 solution with LiOH to the first end point at ~pH 5.6.

The films of the polymer systems were prepared on silicon supports from 0.035 N, 2% pyridine containing aqueous solutions of (L-glu)$_n$ and the respective phosphate in the ratio desired. The precipitation of the polymer films was performed with the centrifugation-drying procedure described in reference 11, whereby instead of a laboratory centrifuge, an ultracentrifuge was used, which improves the quality of the polymer films.

Like the IR cells described in reference 12, the cells used could be evacuated, permitting adjustment of a well defined humidity at the films by use of saturated aqueous solutions of different salts. The IR measurements were performed with an IR spectrophotometer (model 325; Bodenseewerk Perkin-Elmer, Überlingen, FRG).

RESULTS AND DISCUSSION

Fig. 1 shows the spectra of (L-glu)$_n$ + Li^+-, Na^+-, and K^+-dihydrogen phosphate systems as a function of the P_i/glu residue ratio. In Fig. 2 the intensity of the IR continua at 1,900 cm^{-1} is shown as a function of this ratio.

These figures show that with increasing P_i/glu ratio intense continua arise. Fig. 2 shows that the intensity of these continua increase in proportion to the P_i/glu ratio up to a P_i/glu ratio of 5:1, the highest ratio that could be studied.

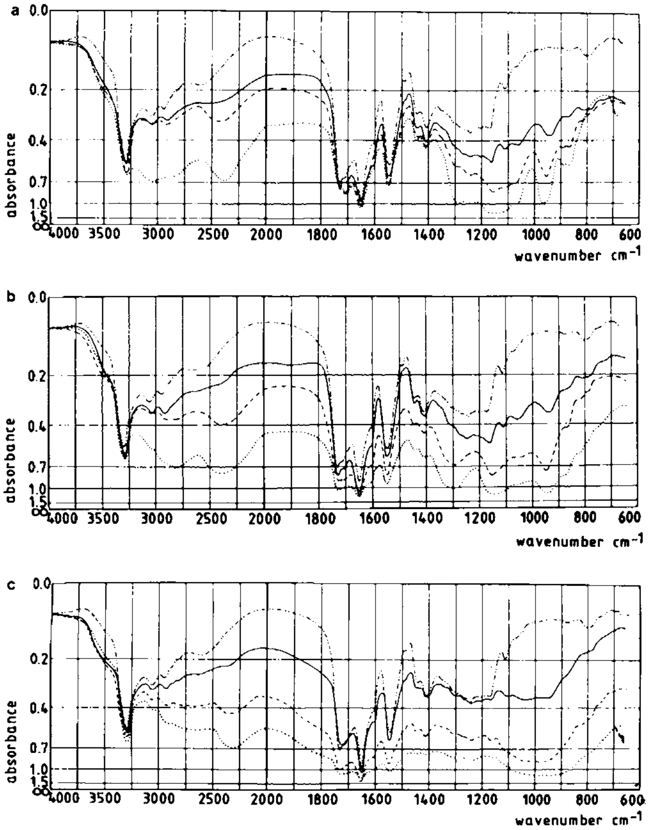


FIGURE 1 IR spectra of dried (L-glu)$_n$ + XH_2PO_4 films with different phosphate/side chain ratios: P_i/glu = 1:3 (——), 1:1 (----), 2:1 (····); pure (L-glu)$_n$ (–···–). Cations present: (*a*) X = Li, (*b*) Na, (*c*) K.

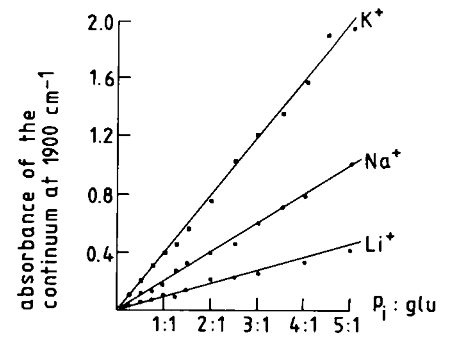


FIGURE 2 Absorbance of the continuum [background in the spectra of (L-glu)$_n$ + XH_2PO_4 minus that in the spectrum of pure (L-glu)$_n$] at 1,900 cm^{-1} of dried (L-glu)$_n$ + XH_2PO_4 systems with various cations.

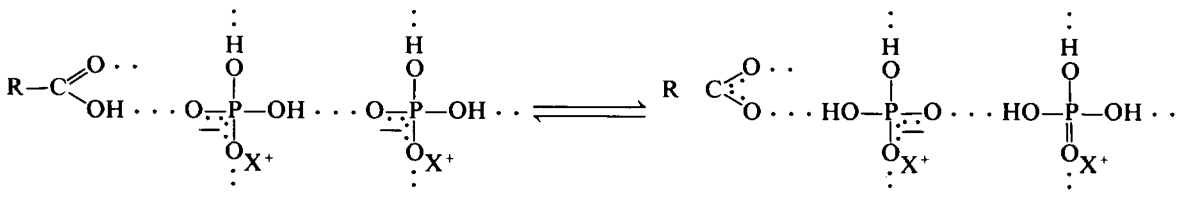
This result demonstrates that the dihydrogen phosphates form chains with the glu residues in which the charge fluctuates and shows large proton polarizabilities due to correlated proton motion (13). The intensity of these continua decreases in the series K^+, Na^+, Li^+ systems. It is known that local fields of cations polarize H-bonds and decrease the proton polarizability of these H-bonds, as indicated by a decrease of the intensity of the continua with increasing electrical cation field (14). Hence, increasing cation fields hinder the charge fluctuation within the chains of hydrogen bonds. This result is confirmed in the following by the behavior of the P_i bands. These chains are particularly stable since the proton motion within these chains proceeds correlated due to proton dispersion forces (15, 16). Caused by these forces the energy is lowered and the chains are stabilized.

With increasing P_i content, ν C=O (C=O stretching vibration) in the region 1,740–1,700 cm^{-1} decreases only slightly. Hence in this proton transfer equilibria the weight of the left proton-limiting structure is much higher than that of the right one.

ν C=O shows, however, characteristic changes as a function of the P_i/glu ratio as well as of the cations present. In the case of the Li^+ systems (Fig. 1 *a*) at 1,710 cm^{-1}, a band arises with increasing P_i/glu ratio. This band is superimposed on the bands of ν C=O of the monomeric (1,730 cm^{-1}) and dimeric (1,710 cm^{-1}) glu residues. It demonstrates that the phosphate chains are bound via strong H-bonds to C=O groups of neighboring glu side chains, as shown in Scheme II.

In the case of the K^+ systems the ν C=O band arises at slightly higher wave numbers, and this band is relatively broad (Fig. 1 *c*). This result shows that the POH· · ·O=C bonds are weaker. This is understandable since with the K^+ system the OH group of P_i is a less stronger donor.

The phosphate bands confirm that the charge fluctuates within these hydrogen-bonded chains. In the P_i/glu, 1:3, K^+ system (Fig. 1 c, *solid line*) only ν_{as} (antisymmetrical stretching vibration) and ν_s (symmetrical stretching vibration) of the P—$(OH)_2$ are observed as weak shoulders at ~970 and 890 cm^{-1}, respectively. The vibrations of the



$X^+ = Li^+, Na^+, K^+$.

Scheme I

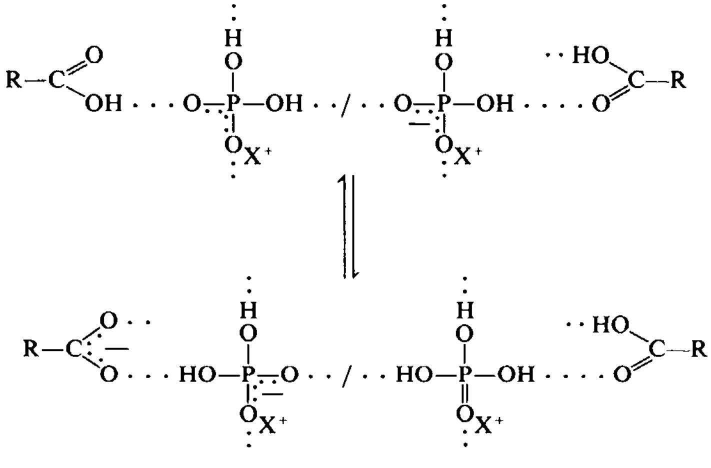
PO_2^- groups in which the PO bands have more double bond character cause a broad band in the region 1,200–1,000 cm^{-1}, which shows only two less pronounced maxima. With increasing P_i content all PO bands in the region melt to one broad absorption in the region 1,200–700 cm^{-1}. To this absorption also contributes the vibration with ν C—O and δ OH (bending vibration) character, found with $(L\text{-glu})_n$ as a band at 1,170 cm^{-1}.

For the Li^+ and Na^+ systems (Fig. 1, *a* and *b*), ν_{as} and ν_s of the $P—(OH)_2$ groups are observed at 940 cm^{-1} and at ~875 cm^{-1}, respectively. These vibrations are still found as bands at higher P_i contents, whereas in the Li^+ system ν_{as} has been shifted a little toward higher wave numbers. With increasing P_i content the bands of ν_{as} and ν_s of the PO_2^- group as well as the vibration with ν C—O and δ OH character are observed as broad band complex in the region 1,200–1,000 cm^{-1}, which shows a maximum at ~1,160 cm^{-1}. For the Li^+ system a shoulder at 1,170 cm^{-1} is still observed probably caused by ν_s PO_2^-. This characteristic cation dependence is illustrated in the case of the P_i/glu, 2:1 systems (Fig. 3). The increase of the proton polarizability is largest going from the Na^+ to the K^+ system. The most pronounced change regarding the melting of the bands occurs between the Na^+ and the K^+ system.

An intense 2 δ OH band (overtone of the OH bending vibration) (12, 17, 18) arises (see Fig. 1) with increasing P_i/glu ratios at 2,420 (Li^+ system), 2,400 (Na^+ system), and 2,350 cm^{-1} (K^+ system). With increasing P_i content this band shifts slightly toward smaller wave numbers. The respective ν OH stretching vibration is found as a broad band with maximum at 2,920 (Li^+), 2,810 (Na^+), and 2,720 cm^{-1} (K^+). The respective δ OH vibration (bending vibration) is observed (see Fig. 3) in the spectra of the 2:1, P_i/glu systems, at ~1,280 cm^{-1} (Li^+) and at 1,300 cm^{-1} (Na^+). In the case of the K^+ systems already at a P_i/glu ratio of 2:1 this band is so strongly broadened that it can no longer be recognized. These bands are characteristic for phosphate–phosphate hydrogen bonds in which the proton is localized at one P_i (12, 17, 18). Thus, these bands show that the chains with fluctuating protons and large proton polarizabilities are cross-linked via $POH\cdots{}^-OP$ hydrogen bonds in which the proton is almost localized at one P_i and shows no noticeable proton polarizability.

INFLUENCE OF HYDRATION

In Fig. 4 the spectra of the hydrated films are compared with the dried ones. In Fig. 5 the intensity of the continua at 1,900 cm^{-1} of films hydrated at 75% air humidity is shown as a function of the P_i/glu ratio. The comparison of Fig. 5 with Fig. 2 shows no considerable change of the



$X^+ = Li^+, Na^+, K^+$.

Scheme II

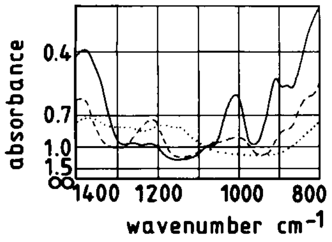


FIGURE 3 IR spectra of dried (L-glu)$_n$ + XH_2PO_4 films (P_i/glu = 2:1) with various cations: X = Li (——), Na (----), K (· · · ·).

intensity of the continuum with increasing degrees of hydration in the region of P_i/glu ratios < 1.5:1. In the case of higher P_i/glu ratios the slope of the curves in Fig. 5 decreases, however, more and more with increasing P_i/glu ratio. The intensity of the continua reaches a limiting value at P_i/glu ratios 4:1 to 5:1. At these P_i/glu ratios the intensity of the continua at 1,900 cm^{-1} is considerably smaller than in the case of the dried systems. Herewith, the influence of the water molecules on the K^+ systems is largest. This result is understandable since the proton polarizability of the chains is largest with the K^+ systems and therefore the interaction of the water molecules with the polarizable chains is largest. The decrease of intensity may have two reasons: First, the water molecules may destroy the hydrogen-bonded chains and reduce in this way the length of the hydrogen-bonded chains. A second reason may be the interaction of the local fields of the hydration water molecules with the hydrogen-bonded chains with large proton polarizability. Caused by this interaction the charge fluctuation is hindered and hence the intensity of the continuum and the proton polarizability of the hydrogen-bonded chains is reduced.

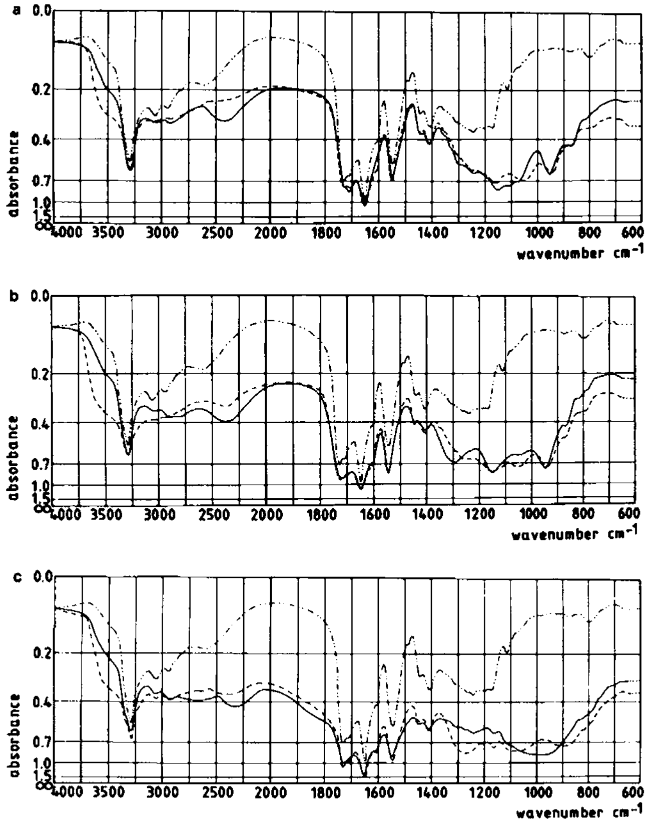


FIGURE 4 IR spectra of (L-glu)$_n$ + XH_2PO_4 films (P_i/glu = 1:1) thoroughly dried (——), at 75% relative humidity of the air (----); pure dried (L-glu)$_n$ (– · · –); cations present; (*a*) X = Li, (*b*) Na, (*c*) K.

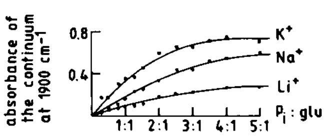


FIGURE 5 Absorbance of the continuum [background in the spectra of (L-glu)$_n$ + XH_2PO_4 minus that in the spectrum of pure (L-glu)$_n$] at 1,900 cm^{-1} of (L-glu)$_n$ + XH_2PO_4 systems at 75% relative humidity; with various cations.

Fig. 4 shows that the band of the 2 δ OH vibration (overtone of the bending vibration), indicating H-bonds between the P_i of the chains in which the proton is almost localized at one P_i, is observed with all systems at ~2,400 cm^{-1}, but in the hydrated systems the intensity of this band has already decreased strongly. This decrease is particularly pronounced with the Li^+ system. The P_i/glu, 2:1 systems, hydrated at 75% relative air humidity are compared with the respective dried samples in Fig. 6. In this case the respective δ OH vibration (bending vibration) at 1,280 cm^{-1} (Li^+ system) and at 1,300 cm^{-1} (Na^+ system) vanishes with hydration. This result shows that the H-bonds formed between the phosphate chains with fluctuating charge are broken to a large extent by the water molecules. In Fig. 6 with the hydrated 2:1 systems, the δ OH vibration of POH· · ·OH_2 bonds is observed, in the case of the K^+ system, as an intense band at 1,270 cm^{-1}. With the Na^+ system this vibration is found as a broad

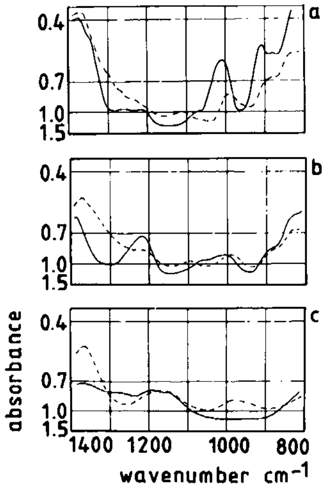


FIGURE 6 IR spectra of (L-glu)$_n$ + XH_2PO_4 films (P_i/glu = 2:1) thoroughly dried (——), at 75% relative humidity (----); cations present: (*a*) X = Li, (*b*) Na, (*c*) K.

shoulder, which is slightly shifted toward smaller wave numbers, and with the Li^+ system, this vibration is observed as a very broad shoulder in the same region. All these results taken together show that with increasing degrees of hydration the H-bonds between the chains with fluctuating charge are broken and water molecules are inserted between the chains.

The result that the charge fluctuation and hence the proton polarizability is reduced caused by the presence of water molecules is confirmed by the behavior of the PO bands. Fig. 6 shows that the spectral feature in the region in which the ν PO vibrations occur changes caused by addition of water molecules. If water molecules are present, well-defined bands of ν_{as} PO_2^-, ν_s PO_2^-, ν_{as} P—$(OH)_2$, and ν_s P—$(OH)_2$ are observed with the Li^+ system at 1,155, 1,055, 940, and 970 cm^{-1}. For the P_i/glu, 2:1, K^+ system the broad absorption of the dried system in the region 1,200–700 cm^{-1} obtains also a structure due to hydration. Two broad absorptions are observed, one in the region 975–1,150 cm^{-1} and one in the region 800–975 cm^{-1}. The first one is caused by the stretching vibrations of the PO_2^- groups and the latter one by the stretching vibrations of the P—$(OH)_2$ groups.

All these changes of the PO bands due to hydration confirm that the fluctuation of the charge in the chains (Scheme II) is slightly hindered due to the presence of water molecules in the systems.

SECONDARY STRUCTURE OF THE (L-GLU)$_n$-DIHYDROGEN PHOSPHATE SYSTEMS

Independent of the cations and the P_i/glu ratio, amide A is found as a relatively narrow band at 3,290 cm^{-1}. Amide I is found at 1,651 and amide II at 1,547 cm^{-1}. Both bands are also relatively narrow. Thus, (L-glu)$_n$ is present as α-helix (19, 20). With hydration these bands do not change. Also in the hydrated systems (L-glu)$_n$ remains a-helical. Hence, the presence of dihydrogen phosphate stabilizes the α-helical structure.

HYPOTHESIS WITH REGARD TO PROTON CONDUCTION IN H^+-ATPase

Finally, our results with the (L-glu)$_n$ + dihydrogen phosphate systems suggest the following hypothesis: The F_0 subunit of the H^+-ATPase in mitochondria is the part of the enzyme that conducts positive charge from the inside of the membrane to the catalytic F_1 subunit (21). Besides other protein molecules, F_0 contains at least six identical, small protein molecules (8 kD, dicyclohexylcarbodiimid binding protein) that are of particular importance for charge conduction (22). It was shown that in these small protein molecules one glutamic acid residue is present in position 58, and this residue is essential for charge conduction (23). With regard to our results with the (L-glu)$_n$ + dihydrogen phosphate systems it seems that it is not impossible that the glu residue of the 8-kD proteins in F_0 form a charge conducting chain with dihydrogen phosphates. Of course, besides such bonds other hydrogen bonds with large proton polarizability between side chains (20) may be of significance for the charge conduction of F_0 (21).

CONCLUSIONS

The behavior of the (L-glu)$_n$ + dihydrogen phosphate systems is completely different compared with the (L-glu)$_n$ + hydrogen phosphate systems. In the latter systems only $COH\cdots{}^-OP \rightleftharpoons CO^-\cdots HOP$ bonds with large proton polarizability are formed (10). For the (L-glu)$_n$ + dihydrogen phosphate systems the glutamic acid groups form chains with at least five phosphates (Scheme II). Within these chains the charge fluctuates and they show very large proton polarizability. The charge fluctuation and, hence, the proton polarizability of the chains increases in the series Li^+, Na^+, K^+ system. All these results are obtained from the intensity of the IR continua as well as from the behavior of the P_i bands as a function of the P_i/glu ratios.

Similar results are obtained with (L-asp)$_n$ + dihydrogen phosphate systems. But with the evaluation of the spectra the difficulty arises that no films of pure (L-asp)$_n$ can be prepared and that the complete formation of the H-bonds is sometimes hindered by sterical reasons.

The comparison of the dihydrogen phosphate with the hydrogen phosphate systems (reference 10) shows that the behavior of (L-glu)$_n$ + phosphate systems strongly changes as a function of the pH. With decreasing pH the glu residues form with P_i hydrogen-bonded chains having large proton polarizability. Thus, the behavior of such systems can be controlled by the pH. In addition the charge fluctuation within these chains can be controlled by the cations present.

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