Uptake of Amino-acids by Zirconium Phosphate. Part 3.† Intercalation of L-Histidine, L-Lysine, and L-Aginine by γ-Zirconium Phosphate

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The uptake of L-histidine (His), L-lysine, and L-arginine by γ-zirconium phosphate has been studied. The intercalation of His is initiated by the partial replacement of interlayer water by a monolayer of the guest molecules oriented horizontally to the sheet of the host crystal to form a phase $Zr(HPO_4)_2(His)_{0.2}\cdot 1.9H_2O$ with an interlayer spacing of 14.1 Å. This is followed by the formation of a phase $Zr(HPO_4)_2(His)_{0.4}\cdot 0.8H_2O$ without any appreciable change in interlayer spacing, which is finally converted into a phase $Zr(HPO_4)_2(His)_{0.9}\cdot 3H_2O$ with an interlayer spacing of 22.9 Å in which the guest molecules are intercalated as a bilayer. Lysine and arginine also form one or two monolayered phases at low uptakes, while at higher loadings the intercalates formed are highly disordered, but with a certain degree of order in the host lattice. The different behaviour of α- and γ-zirconium phosphates in intercalating amino acid molecules is discussed in terms of the structural or functional characteristics of the individual acids and phosphates. A supplementary discussion of the amino acid arrangement in α-zirconium phosphate is also made.

Layered zirconium phosphate can be obtained in two representative forms: the α phase, $Zr(HPO_4)_2\cdot H_2O$, with an interlayer spacing of 7.6 Å, and the γ phase, $Zr(HPO_4)_2\cdot 2H_2O$, with an interlayer spacing of 12.3 Å.[1] These two phases behave as intercalating agents for polar substances.[2] Although much attention has been paid to the intercalation behaviour of α-zirconium phosphate towards various compounds, there has been little interest in a comparative study on the intercalation properties of α- and γ-zirconium phosphates.[2] A striking contrast between these two phases as host matrices was revealed by the observation that the pyridine intercalate of γ-zirconium phosphate releases the guest molecules at temperatures more than 100 °C higher than that of the α compound.[3]

In a previous paper on the uptake of basic amino acids by α-zirconium phosphate we showed that the intercalation behaviour of L-lysine (Lys) contrasts markedly with those of L-histidine (His) and L-arginine (Arg).[4] An attempt was thus made to investigate the intercalation behaviour of γ-zirconium phosphate towards His, Lys, and Arg.

Experimental

The γ-zirconium phosphate sample was the same as that used elsewhere.[3] L-Histidine, L-lysine, and L-arginine were reagent grade and used without further purification.

The equilibrium procedure was identical with that described previously.[4] The phosphate sample (0.1 g) was suspended in distilled water and a predetermined amount of 0.2 mol dm^{-3} amino acid solution was added to keep the volume to solid ratio constant at 40 $cm^3\ g^{-1}$. After shaking at 25 ± 0.5 °C for 4 d, the solids were separated and stored in a desiccator over calcium nitrate solution (relative humidity 0.51) at 25 °C. The amino acid and phosphate concentrations and the pH of the supernatants were measured as previously.[4] The resulting solids were characterized by room-temperature *X*-ray diffraction, electromicroscopy, i.r. absorption, and thermogravimetry (t.g.) as described previously.[4]

The phosphate complexes with histamine and pentamethylenediamine were prepared similarly. However, only the numerical data necessary for a comparative study are cited.

† For Part 2 see ref. 4.

Results

The amino acid uptakes *x*, in moles per formula weight of phosphate, are plotted against the amount added in Figure 1. In contrast to α-zirconium phosphate,[4] all the curves for uptake of His, Lys, and Arg into γ-zirconium phosphate show only one plateau at levels greater than 4 mmol g^{-1} and the maximum uptakes for these amino acids are 2.85 (x = 0.91), 3.20 (1.02), and 2.60 mmol g^{-1} (0.83), respectively. Figure 2 shows the variation of the pH of the equilibrated solution in the uptake process.

As shown in Figure 3, the uptakes of Lys and Arg at levels of greater than 2 mmol g^{-1} were accompanied by a remarkable increase in the release of phosphate due to the hydrolytic effect of the basic functions, while the phosphate release for His was kept at a level as low as 0.04 mmol g^{-1}.

The linear phase diagrams for the amino acid complexes determined by *X*-ray diffraction are shown in Figure 4. At low uptakes, Lys formed two intercalated phases (I_1 and I_2) whose interlayer spacings are *ca.* 14 and 16.0 Å, while the other two amino acids yielded only one intercalated phase (I_1) with an interlayer spacing of 14.1 (His) or 13.5 Å (Arg). These intercalated phases, except for the I_2 phase for Lys, coexisted with their host phase, γ-zirconium phosphate, at $x \leqslant 0.13$, *cf.* $x \leqslant 0.45$ for coexistence of α-zirconium phosphate and its complexes containing the same amino acids. When $x \geqslant 0.47$ for Lys and $x \geqslant 0.25$ for Arg the intercalated solids became progressively disordered and less regular in particle shape until

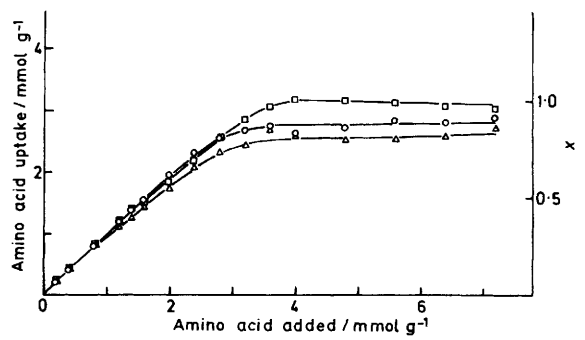

Figure 1. Amino acid uptake by γ-zirconium phosphate as a function of the amount added: ○, His; □, Lys; △, Arg

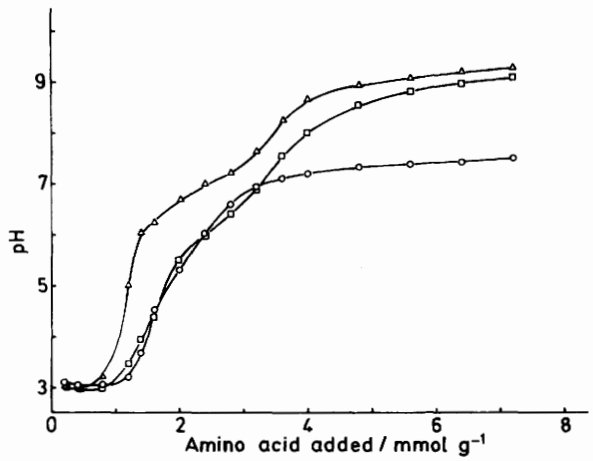

Figure 2. Potentiometric titration curves for γ-zirconium phosphate with His (○), Lys (□), and Arg (△)

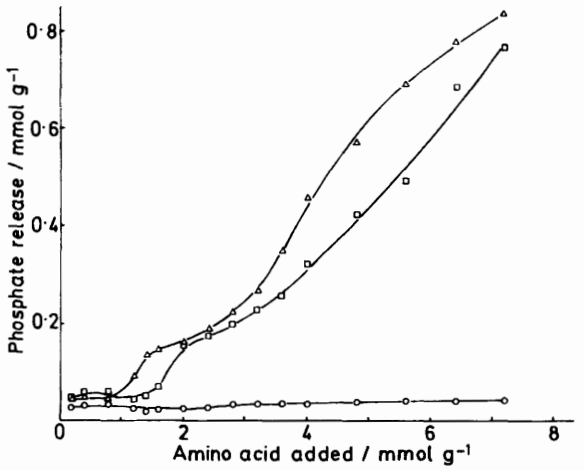

Figure 3. Millimoles of phosphate released to the solution per gram of γ-zirconium phosphate. Key as in Figure 2

at $x \geqslant 0.69$ for Lys and $x \geqslant 0.74$ for Arg the solids were formed of amoebiform aggregates, the *X*-ray patterns of which showed one sharp peak at $d = 3.3$ Å and two other weak peaks (Table 1). Histidine, on the other hand, induced no significant disorder and the intercalated particles retained their original ribbon shape. While the diffraction peaks for the 14.1-Å phase commenced to decrease in intensity at $x = 0.38$, a new intercalated phase (I_2) with interlayer spacing 22.9 Å appeared at $x = 0.6$ and developed with a further increase in uptake until when $x \geqslant 0.74$ only the latter phase was observed. The intercalation of His without significant disorder would serve effectively to hamper the release of phosphate. This is consistent with the observed behaviour.

Further detailed investigation using thermogravimetry was carried out on the γ-zirconium phosphate complexes with His. T.g. curves for several of the His-intercalated solids are shown in Figure 5. The first weight loss at below 150 °C is due to desorption of water. This was followed by loss of the amino acid which commences at about 210 °C and the final product was α-ZrP_2O_7. Therefore, the second weight loss in the temperature range 210—1 000 °C can be ascribed to decomposition of the amino acid and condensation of phosphate groups. The His (x) and water (y) contents in the intercalate, expressed in moles per formula weight of intercalate, were determined from the t.g.

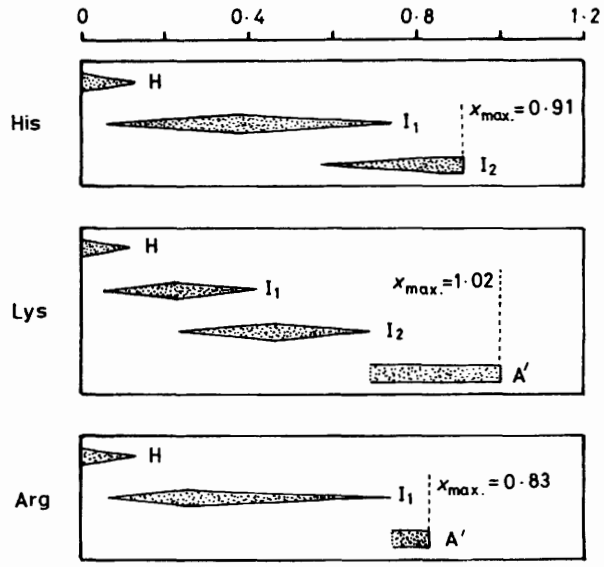

Figure 4. Linear phase diagram for γ-zirconium phosphate complexes with His, Lys, and Arg: H = host phase; I_i(i = 1 or 2) = intercalated phases; A′ = pseudo-amorphous phase (see text). Interlayer spacings (Å) of phases I_1 and I_2: His 14.1, 22.9; Lys *ca.* 14, 16.0; Arg 13.5, —. The increase or decrease in the phase composition is indicated schematically by a bar of increasing or decreasing width and the maximum value is denoted as $x_{max.}$

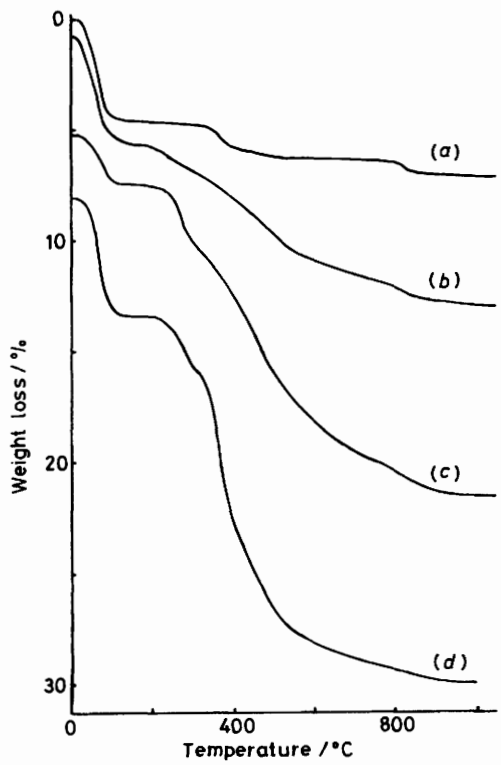

Figure 5. T.g. curves for the His intercalates of γ-zirconium phosphates at a heating rate of 10 °C min^{-1}. Amounts (mmol g^{-1}) of amino acid added: 0 (*a*), 0.4 (*b*), 1.6 (*c*), and 7.2 (*d*)

data and are shown in Figure 6. It is interesting that the y *vs.* x curve shows a plateau in the range of $0.13 \leqslant x \leqslant 0.2$ and a minimum at $x \approx 0.4$. This indicates that the 14.1-Å phase for

Table 1. *X*-Ray diffraction data for the (*a*) Lys and (*b*) Arg intercalates of γ-zirconium phosphate separated at the stage of addition of 7.2 mmol amino acid per gram of phosphate. Data for the Lys intercalate of α-zirconium phosphate separated at 3.2 mmol g^{-1} are also given in (*c*)[4]

(*a*)			(*b*)			(*c*)		
hkl	*I*	*d*/Å	*hkl*	*I*	*d*/Å	*hkl*	*I*	*d*/Å
020	s	3.29	020	s	3.31	110	vw	4.52
	vw	2.03	200	vw	2.68	020	m	2.64
040	vw	1.69	040	vw	1.65	040	vw	1.33

Table 2. *X*-Ray diffraction data for the His intercalate separated at the stage of addition of 7.2 mmol His per gram of γ-zirconium phosphate

d/Å	I/I_0	*d*/Å	I/I_0
22.9	100	3.19	6
11.5	21	3.09	7
6.10	15	2.69	7
5.39	20	2.67	7
4.61	11	2.65	4
4.15	18	2.47	5
3.92	5	2.19	5
3.69	18	2.15	3
3.32	34	2.08	5
3.29	33		

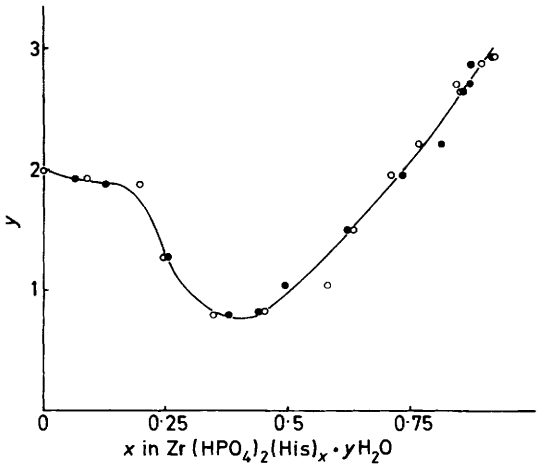

Figure 6. Plots of the water content (*y*) against amino acid uptake (*x*) for the His intercalate of γ-zirconium phosphate, $Zr(HPO_4)_2(His)_x \cdot yH_2O$. The *x* values were determined from the t.g. (○) or solution analysis (●) data

His, I_1, is subdivided into a phase (I_{1a}), $Zr(HPO_4)_2(His)_{0.2} \cdot 1.9H_2O$, formed near $x \approx 0.2$ and a phase (I_{1b}), $Zr(HPO_4)_2(His)_{0.4} \cdot 0.8H_2O$, formed near $x \approx 0.4$. The 22.9-Å phase for His, on the other hand, can be regarded as having the composition $Zr(HPO_4)_2(His)_{0.9} \cdot 3H_2O$. The *X*-ray diffraction data for this phase are given in Table 2.

The i.r. spectra of the γ-zirconium phosphate complexes with His, Lys, and Arg were similar to those for the α-zirconium phosphate complexes.[4] In each spectrum for the former complexes separated from the solution at low pH, a weak band attributable to the C=O stretching mode of the COOH group was observed near 1 740 cm^{-1}. For the complexes obtained at higher pH this band showed no appreciable increase in intensity (Lys) or disappeared completely (His and Arg). Considering the dissociation properties of these amino acids, it is suggested that the carboxyl groups of the intercalated molecules have the same ionized or un-ionized form as in the equilibrated solution, as observed for the α complexes.[4]

Discussion

In the previous study[4] the mechanism of intercalation of His, Lys, and Arg into α-zirconium phosphate was discussed on the basis of the dissociation and structural properties of these amino acids and the i.r. and *X*-ray data for the intercalated phases. A supplementary discussion based on the crystal structure of α-zirconium phosphate is made in the Appendix. Here we compare the intercalation behaviour of these amino acids for γ- and α-zirconium phosphate.

It is reasonable to assume that the γ-phosphate crystal also intercalates molecules of His, Lys, and Arg by replacement of its interlayer surface protons by the protonated α-amino-, ε-amino-, and guanidino-groups, respectively. At low uptake the intercalation results in the formation of a solid in which the interlayer space is occupied by a monolayer of the guest molecules located nearly parallel to the phosphate layer or in slightly more upright orientation. This is because the interlayer spacings of the I_1 and/or I_2 phases are larger than 9.4 Å for the anhydrous form of their host phase, β-zirconium phosphate,[1] respectively, by 4.7 Å for His, 4.6 and 6.6 Å for Lys, and 4.1 Å for Arg, fairly close to the van der Waals thickness (4.3—4.4 Å) of the amino acid molecules. Furthermore, the *X*-ray diffraction peak at $d = 3.3$ Å observed for the highly disordered intercalates of Lys and Arg corresponds to the (020) reflection of γ-zirconium phosphate as indexed by Yamanaka and Tanaka[5] (Table 2). This fact appears to suggest that at low uptakes the amino acid molecules are likely to be intercalated with their long axes parallel to the *a* axis of the host crystal, as observed in α-zirconium phosphate. However, since the structure of γ-zirconium phosphate has not yet been solved, no further detailed comments can be made on the molecular arrangement of the monolayered phase of γ-zirconium phosphate. The water molecules in the γ-phosphate are weakly held by interlayer hydrogen bonding to the POH sites.[6] For His, the parallel placement of the guest molecules is likely to be accompanied by the release of as little as 0.1 mol of interlayer water per formula weight, yielding phase I_{1a}. This would be followed by the formation of phase I_{1b} in which a further 1.1 mol of interlayer water per formula weight are replaced by 0.2 mol of His without any appreciable change in interlayer spacing. A similar observation was made for the intercalation of pyridine into the γ-phosphate.[3]

The intercalation behaviour of His, Lys, and Arg into γ-zirconium phosphate at higher loadings are strikingly different from that with the α-phosphate.[4] Table 3 summarizes the intercalation parameters for His, Lys, Arg, histamine (Hist), and pentamethylenediamine for α- and γ-zirconium phosphates. Histamine and pentamethylenediamine do not possess a carboxyl group but have the same basic skeleton as His and Lys, respectively. α-Zirconium phosphate formed a crystalline bi-ation behaviour of pentamethylenediamine with γ-zirconium phosphate was similar to that with the α-phosphate, probably

Table 3. Interlayer spacing and composition of intercalates of α-and γ-zirconium phosphates with basic amino acids and related amines at their full loadings. The data referring to the α-intercalates are taken from ref. 4

			α-Intercalate				γ-Intercalate			
Intercalant	δ_1[a] Å	δ_2[b] Å	Interlayer spacing/ Å	Δ[c]/ Å	Composition[d]	Type of intercalate[e]	Interlayer spacing/ Å	Δ[c]/ Å	Composition[d]	Type of intercalate[e]
His	10.5	4.4	—	—	1.2	A	22.9	13.5	0.9	Bilayer
Lys	12.1	4.3	23.1	15.7	1.85	Bilayer	—	—	1.0	A′
Arg	13.1	4.3	—	—	1.2	A	—	—	0.8	A′
Hist	10.1	4.0	20.0	12.6	2.0	Bilayer	14.3	4.9	0.6	Monolayer
pmda[f]	11.4	4.0	15.5	8.1	1.0	Bilayer	18.4	9.0	1.0	Bilayer

[a] Maximum molecular length. [b] Minimum molecular thickness. [c] Obtained by subtracting 7.4 and 9.4 Å from the observed spacings for the α- and γ-intercalates, respectively. [d] Mol of intercalant per formula weight of host phosphate. [e] A = Amorphous phase; A′ = pseudo-amorphous phase (see text). [f] Pentamethylenediamine.

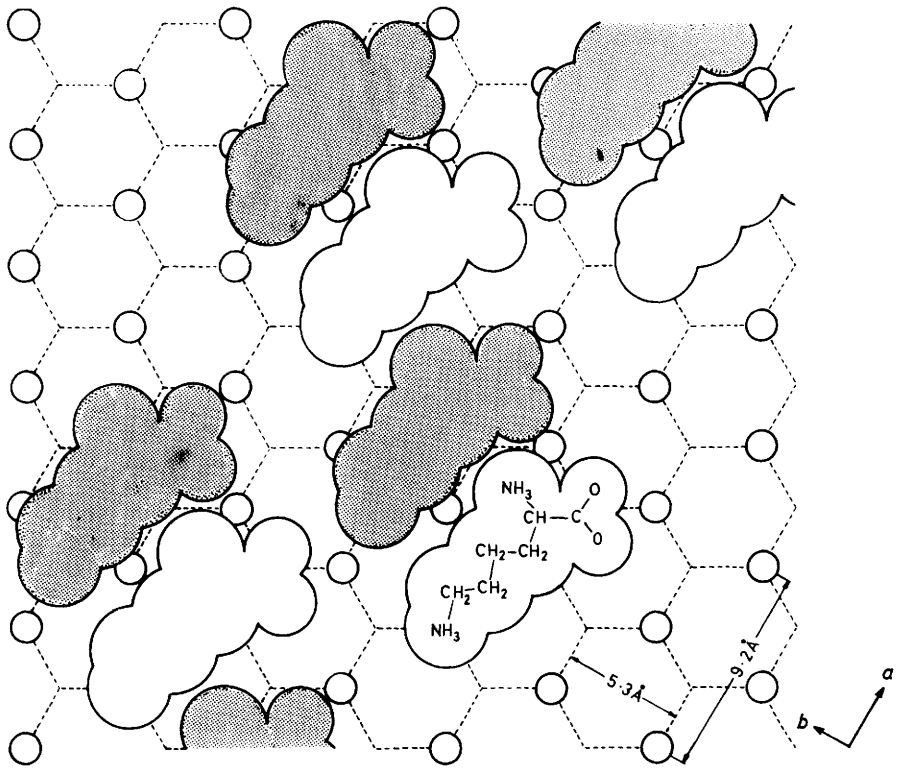

Figure 7. A possible arrangement of Lys in the monolayered phase of α-zirconium phosphate. The Lys molecules are bonded to the lower and upper (shaded) layers and lie along the *a* axis. Circles represent the POH groups at the surface of the lower layer

on the other hand, the His intercalate formed at high loadings is a bilayered crystal in which the skeletal chain makes an angle of *ca.* 45° with the phosphate sheet, while the other two intercalates are highly disordered but their *X*-ray diffraction patterns are indicative of a certain degree of order in the host lattice. In the previous paper,[4] whether the bilayered phases of α-zirconium phosphate with His, Lys, and Arg are disordered or not was attributed primarily to the structural characteristics of the individual acids, that is whether the groups through which the amino acid molecules are anchored to the POH sites are free from any neighbouring groups such as carboxyl. In γ-zirconium phosphate the intercalated molecules are likely to be much more subject to steric hindrance than in α-zirconium phosphate because the POH sites in the former, available for the bonding of guest molecules to the interlayer surface, are arranged more compactly than in the latter.[5,7] Therefore, the disorder of the Lys and Arg intercalates of γ-zirconium phosphate could be explained by assuming that the carboxyl and amino termini of the guest molecules are sterically hindered as a result of the spatial restrictions by the host lattice. On the other hand, the bilayered intercalation of His by γ-zirconium phosphate is due to the structural compatibility of the molecule with the interspace characteristic of the host phase. Such molecular structure selectivity of γ-zirconium phosphate as a host compound was also observed with Hist. Histamine formed only a monolayered intercalate with γ-zirconium phosphate, but a bilayer with α-zirconium phosphate (Table 3); further details will be reported elsewhere. In contrast to Hist, the intercalation behaviour of pentamethylenediamine with γ-zirconium phosphate was similar to that with the α-phosphate, probably

due to the bifurcated bonding of both terminal amino groups to the POH sites in either host crystal.

Appendix

α-Zirconium phosphate possesses a layer structure,[1] each layer consisting of a plane of zirconium atoms bridged through phosphate groups located alternately above and below this plane. Three oxygen atoms of the tetrahedral phosphate group are bonded to three zirconium atoms in the plane and the fourth oxygen atom bears a hydrogen and points toward an adjacent layer in the structure. The POH groups pointing up or down in each layer surface are located in a monoclinic cell with distances of 9.2 and 5.3 Å along the *a* and *b* axes, respectively. Any two adjacent layers are staggered in such a way as to place the POH groups directly below the zirconium atom (and *vice versa*) in the layer above. Furthermore, the highly disordered intercalate of α-zirconium phosphate with Lys at $x = 0.4$—1.0 showed a strong diffraction peak attributable to the (020) reflection of the host crystal (Table 2) and this diffraction peak was also shown by the His and Arg intercalates in their highly disordered states. These *X*-ray observations may be explained by assuming that the amino acid molecules (maximum length 10.5—13.1 Å) bonded to any two adjacent phosphate layers above or below are arranged alternately along the *a* axis, because the phosphate layers bearing the uniaxially oriented guest molecules would be displaced preferentially along this axis. Thus, for the monolayered phase formed at low uptakes it is suggested that the amino acid molecules with their long axes parallel to the *a* axis are anchored through the basic termini[4] to every second POH site in every fourth row along this axis in each layer surface, as shown for Lys in Figure 7. This arrangement yields a maximum *x* value of 0.25. As the loading is increased further the amino acid molecules may be forced into a more upright position, which would induce disorder in the packing sequence of the phosphate layers and/or the atomic arrangements within each layer.[4] This would account for the *X*-ray results, *i.e.* the phase compositions of the monolayered solids (I_1 for His, I_2 for Lys, and I_1 for Arg in ref. 4) reach a maximum when $x \approx 0.25$ and, with a further increase in the amino acid loading, the intercalated solids exhibit an increase in interlayer spacing, followed by increased disorder or decrystallization for Lys and Arg.

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