[Chem. Pharm. Bull. 28(5)1491—1496(1980)]

Physicochemical Studies on Calcium Glycerophosphate. I. X-Ray Diffraction Study of Calcium Glycerophosphate Crystals and Their Water of Crystallization

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(Received October 24, 1979)

Calcium *dl*-α-glycerophosphate (α-CaG) has three crystal forms (anhydrate, mono- and di-hydrates), and calcium β-glycerophosphate (β-CaG) has two (anhydrate and monohydrate). The X-ray diffraction patterns of these crystals show a characteristic strong peak at a Bragg angle (2θ) of 6° to 8°. This peak is very sensitive to de- and rehydration of the hydrate crystals; for α-CaG hydrates, the 2θ angle of the peak shifts, while that of β-CaG hydrate does not change, but its intensity changes. Since the dehydrated CaG hydrates recover their original hydrate forms at appropriate relative humidities without any crystal damage, the rate constants of rehydration were measured using the gravimetry and X-ray intensity methods. β-CaG monohydrate has a smaller value of the rate constant than the α-CaG dihydrate, indicating that the dehydrated crystals of the former are more stable than those of the latter, and that water of crystallization in the former may not play as important a role in crystal growth as in the latter. As for α-CaG dihydrate, the rate of rehydration through the incorporation of water into the dehydrated crystal is smaller than the total velocity of water gain (involving water of crystallization and adsorbed water), suggesting that the water molecule is first adsorbed onto the dehydrated crystal and is then incorporated into the crystal. The formation of the different CaG crystals may be a result of differences in the mode of interaction between the water molecules and calcium ions.

Keywords——calcium *dl*-α-glycerophosphate; calcium β-glycerophosphate; X-ray diffraction pattern; rate constant; rehydration; dehydration; pseudo first-order reaction

Calcium glycerophosphate (hereafter abbreviated as CaG) is used medically as a source of calcium and phosphate for the body. It is also of interest because it is a constituent of phospholipid molecules,[2] which are major components of biological cell membranes. Structural studies on the interation between glycerophosphate and calcium ions might provide some insight into the transport of metal ions through membranes.

The binding mode of calcium with glycerophosphate seems to be different from that of such other metal ions as sodium[3] and cadmium[4]; CaG has five crystal forms, consisting of *dl*-α-CaG (anhydrate, mono- and di-hydrates) and β-CaG (anhydrate and monohydrate). The crystals show characteristic X-ray diffraction patterns having the strongest peak near a Bragg angle (2θ) of 6° to 8°.[5]

The present paper deals with the preparation of CaG crystals, the characteristics of their X-ray diffraction patterns and their thermal behavior as regards water of crystallization.

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Experimental

Preparation of CaG Crystals——Sodium *dl*-α- and β-glycerophosphate were purchased from Sigma Co. (U.S.A.) and were dissolved in water. An equimolar amount of calcium chloride was added to the solution at a defined temperature as shown in Table I, and the mixture was stirred for 2 hr (the time required to precipitate CaG completely). The precipitate was filtered off and washed using 45% aqueous ethanol solution until no chloride ion was detected with silver nitrate. The α- and β-CaG crystals obtained were more than 96.5% pure as estimated by the periodic acid method.[6]

Determination of the Water Content of Crystals——Total water content (the sum of water of crystallization and adsorbed water) of CaG crystals was determined by the Karl Fisher method. The amount of water of crystallization and the temperature at which it was lost were confirmed by thermogravimetry and differential thermal analysis (using instruments from Rigaku Denki Co., Japan).

X-Ray Diffractometry——Diffraction patterns of α- and β-CaG powders were measured at 25° with an X-ray diffractometer (Rigaku Denki) using Ni-filtered Cu-Kα radiation (at a scan speed of 1°/min. and with a 0.1 mm receiving aperture).

Determination of the Rate Constant of Rehydration of Predehydrated CaG Crystals——The following scheme can be applied to the reaction between CaG and water.

$$\mathrm{CaG} + n\mathrm{H_2O} \rightleftarrows \mathrm{CaG}\cdot n\mathrm{H_2O}$$

This reaction can be regarded as a pseudo first-order reaction, provided that the amount of water is constant. Therefore, the value of the rate constant (k) for rehydration can be determined using the following two methods:

1. Gravimetry

Here we have the relation:

$$k = \frac{2.303}{t} \log \frac{W_\infty - W_0}{W_\infty - W_t} \qquad (1)$$

where W_∞ is the weight upon complete rehydration of the predehydrated CaG crystals, and W_0 and W_t are the weights of the completely dehydrated CaG crystals and the partially rehydrated CaG at time t, respectively.

2. X-ray intensity method

The intensity of a certain reflection from component i in a mixture is given by the following equation.[7]

$$I_i = \frac{C_i X_i}{\rho_i[(\mu_i - \mu_m)X_i + \mu_m]} \qquad (2)$$

where X_i is the weight fraction of component i in the mixture, μ_i is the mass absorption coefficient of the solid component i at the X-ray wave-length used, μ_m is the mass absorption coefficient of the sum of the other components, ρ_i is the density of solid component i, and C_i is a constant depending upon the nature of component i and the geometry of the apparatus.

The mass absorption coefficient of CaG crystals is 44.6 for the dihydrate, 44.9 for the monohydrate and 50.3 for the anhydrate. Provided that these values are regarded as approximately equal, equation (2) gives (3):

$$\mathrm{I}_i = C'X_i \qquad (3)$$

where C' is $C_i/\rho_i\mu_i$.

Equation (3) shows that the degree of rehydration of predehydrated CaG can be monitored in terms of the change of the X-ray diffraction intensity. Therefore, the rate constant can be estimated using equation (4):

$$k = \frac{2.303}{t} \log \frac{H_\infty - H_0}{H_\infty - H_t} \qquad (4)$$

where H is the peak area of a certain strong reflection of CaG crystals at a fixed Bragg angle (2θ), and the subscripts of H have the same meaning as in equation (1).

The rate constant given by equation (4) is only related to the association between CaG and water of crystallization, whereas that given by equation (1) is related to the association between CaG and total water (water of crystallization and adsorbed water).

The sample used for the X-ray measurement was held in the diffraction sample holder during rehydration, and the relative humidity for rehydration was controlled according to the literature.[8]

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Results and Discussion

The Relation between the Water Content in CaG Crystals and the Reaction Temperature

When CaG is synthesized from sodium glycerophosphate and calcium chloride in aqueous solution, the content of water of crystallization is mostly influenced by the reaction temperature, and is not much influenced by other conditions such as concentration and molar ratio. The relation between the content of water of crystallization and the reaction temperature is summarized in Table I. The measurements of water content listed in Table I were done after drying the samples for one day in a desiccator (silica gel) at 25°. Depending on the reaction temperature, α-CaG takes three crystal forms (anhydrate, mono- and di-hydrates), and β-CaG takes two crystal forms (anhydrate and monohydrate).

Table I. Relation between the Amount of Water of Crystallization contained in CaG Crystals and Reaction Temperature

	Temp. (°C)	Crystal water	
α-CaG	$\leqq 2$	H_2O	(8.0)
	10—30	$2H_2O$	(14—15)
	$\geqq 50$	Anhydrate	(<0.2)
β-CaG	$\leqq 15$	H_2O	(8.1)
	$\geqq 17$	Anhydrate	(<0.1)

The values in parentheses show the water content (%) determined by the Karl Fisher method.

All these hydrates except for α-CaG monohydrate were completely dehydrated at 130°, while α-CaG monohydrate was dehydrated at above 150°, as judged by the Karl Fisher method. This suggests that the interaction between water of crystallization and the CaG molecule is stronger in α-CaG monohydrate than in the other forms.

Characteristics of the X-Ray Diffraction Patterns

Figure 1 shows the X-ray diffraction patterns ($4° \leqq 2\theta \leqq 60°$) of the three crystal forms of α-CaG and two crystal forms of β-CaG. In all the crystal forms, the strongest peak appears in the 2θ range of 6° to 8°. These 2θ angles are slightly different from each other, and may be intrinsic to each crystal form.

Upon dehydration of CaG hydrates, the intensities of their diffraction peaks decrease slightly in the 2θ range of 5° to 60°, and the 2θ angles of α-CaG crystals shift. The strongest peaks near at 6° to 8° are very sensitive to re- and dehydration. The changes in the 2θ angles of the peaks at 25° and after drying at 155° are listed in Table II, together with their interplanar distances.

The dehydrated crystals are clearly different from the anhydrate crystals in their three-dimensional structures, because their X-ray diffraction patterns are different.

It is of interest that the interplanar distance corresponding to the strongest peak of α-CaG monohydrate becomes longer on dehydration, while that of the dihydrate becomes shorter. The changes of the diffraction patterns of hydrates on dehydration imply the occurrence of structural change in their crystals, and the increase of the interplanar distance in α-CaG monohydrate on dehydration may be due to loss of the hydrogen bonding between water and α-CaG molecules.

On the other hand, in β-CaG monohydrate, a slight decrease of the X-ray diffraction intensities was observed without any change of the 2θ angle within the diffractional resolution, suggesting that the role of water of crystallization in the growth of the crystal may not be as important as in α-CaG hydrates.

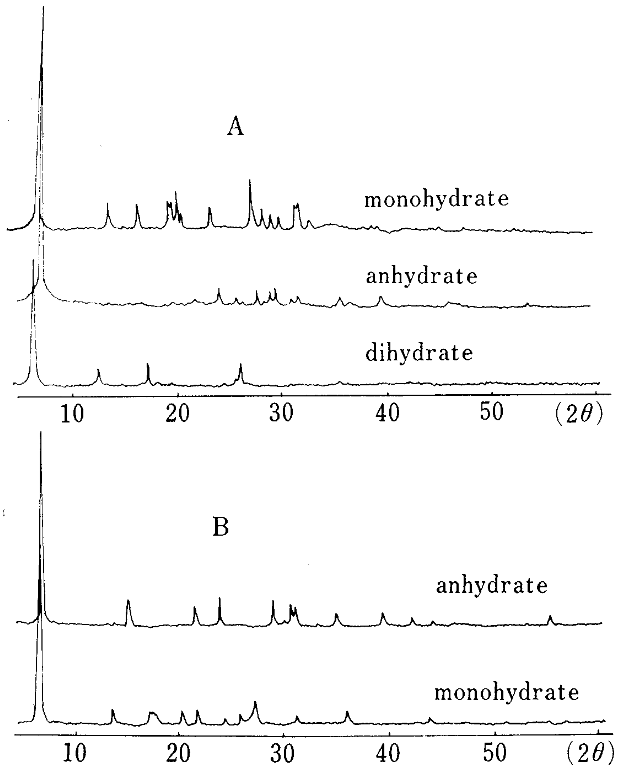

Fig. 1. X-Ray Diffraction Patterns of CaG Crystals

A) α-CaG, B) β-CaG.

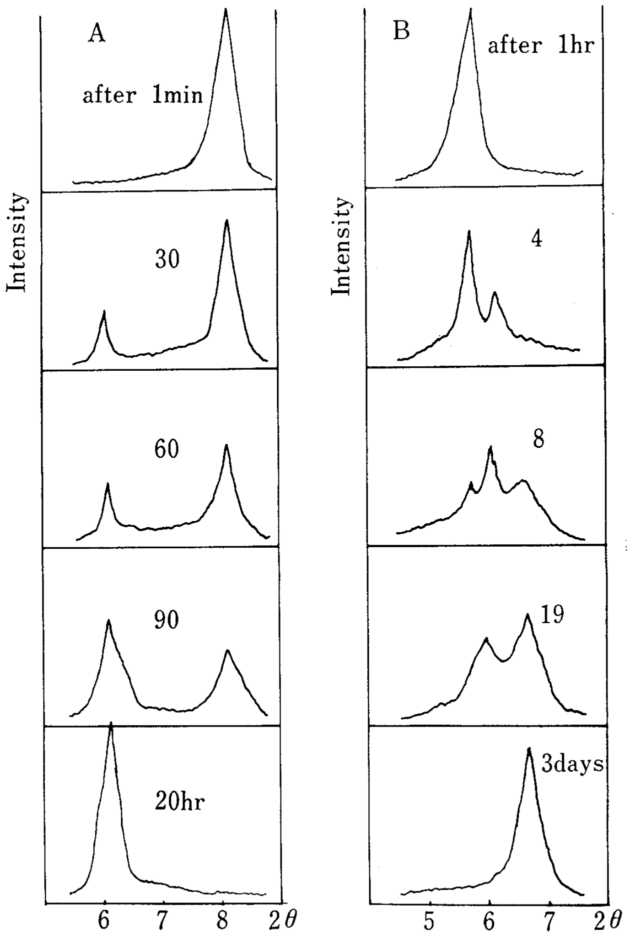

Fig. 2. X-Ray Profile of the Strongest Peak during Rehydration of Dehydrated CaG Crystals

A) α-CaG dihydrate, B) α-CaG monohydrate.

Table II. X-Ray Scattering Angles and the Corresponding Interplanar Distances for the Characteristic Strongest Peaks (see the text) at 25° and after Drying at 155°

CaG crystals	Before heating		After heating	
	2θ(°)	d(Å)	2θ(°)	d(Å)
α-CaG·H_2O	6.6	13.39	5.8	15.24
α-CaG·$2H_2O$	6.1	14.49	8.1	10.92
α-CaG(anhydrate)	7.1	12.45	—	—
β-CaG·H_2O	6.6	13.39	6.6	13.39
β-CaG(anhydrate)	7.0	12.63	—	—

Investigation of the Rehydration Velocity of Dehydratcd CaG Crystals

The dehydrated CaG crystals obtained by heating at 155° recover their original hydrate form on rehydration, as indicated by the X-ray diffraction patterns. In order to investigate their rehydration behavior, we measured the changes in the diffraction patterns of predehydrated CaGs on leaving the samples at a fixed relative humidity and temperature (20°) for an appropriate time.

In the case of predehydrated α-CaG dihydrate, the intensity of the strongest peak ($2\theta=8.1°$) decreased with rehydration time, with a concomitant increase in the intensity of the peak at $2\theta=6.1°$, the strongest peak in the original dihydrate crystals (see Fig. 2).

In the case of predehydrated α-CaG monohydrate, the rehydration differed significantly from that of the dihydrate; the rehydration rate was extremely slow, requiring three days for complete recovery of the original monohydrate form. Further, as shown in Fig. 2, an intermediate peak which is not due to hydrate ($2\theta=6.6°$) or to anhydrate ($2\theta=5.8°$) emerged during rehydration. This suggests that the rehydration process of dehydrated α-CaG monohydrate is complicated (it does not obey pseudo first-order kinetics), and probably involves an unknown phase modification.

In the case of predehydrated β-CaG monohydrate, no change of 2θ angle was observed during rehydration, but the intensity of the strongest peak ($2\theta=6.6°$) increased slightly with the rehydration time

For α-CaG dihydrate, the rehydration rate was measured by the gravimetry and X-ray intensity methods, while, for β-CaG monohydrate, it was measured by gravimetry alone. For β-CaG monohydrate, the rehydration rate measured by the X-ray intensity method was so slow that we could not determine the exact k-value at 93% relative humidity.

Figure 3 illustrated the change during rehydration, and their rate constants (k) are given in Table III under various relative humidities at 20°.[9]

In the gravimetry method, the k-value of β-CaG monohydrate is smaller than that of α-CaG dihydrate at any humidity, suggesting that the dehydrated crystals of β-CaG monohydrate are more stable than those of α-CaG dihydrate, and this may also be consistent with the unchanged angle of the strongest diffraction peak in the process of re- and dehydration.

In the case of the rehydration of dehydrated α-CaG dihydrate, the k-value obtained by the X-ray intensity method is smaller than that obtained by gravimetry at any relative humidity. The quantities measured by the latter method involve not only the water of crystallization but also the adsorbed water, while those measured by the former method involve only

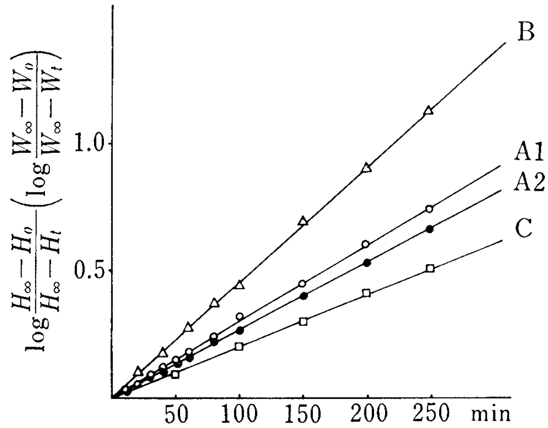

Fig. 3. The Pseudo First-order Reaction for Rehydration at 93% Relative Humidity

A: rehydration of dehydrated α-CaG dihydrate followed by the X-ray intensity method using the strongest peak at $2\theta=8.1°$ (A1) and 6.1° (A2).
B: rehydration of dehydrated α-CaG dihydrate followed by the gravimetry method.
C: rehydration of dehydrated β-CaG monohydrate followed by the gravimetry method.

Table III. Rate Constants for the Rehydration of Dehydrated CaG Hydrates under Various Levels of Relative Humidity at 20°

CaG hydrate	Relative humidity[a] ($k(\times 10^2)\mathrm{min}^{-1}$) 93%	75%	47%	Method
α-CaG·$2H_2O$	0.69	0.39	0.18	X-ray ($2\theta=8.1°$)
	0.63	0.40	0.18	X-ray ($2\theta=6.1°$)
	1.04	1.01	0.36	Gravimetry
β-CaG·H_2O	0.46	0.33	0.22	Gravimetry
	<0.1	—	—	X-ray ($2\theta=6.6°$)

a) Saturated aqueous solutions of $NH_4H_2PO_4$, $NaClO_3$, and KSCN correspond to relative humidities of 93%, 75% and 47% at 20°.[9]

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9) "The Merck Index," 9th Edition, ed. by M. Mindholz, Merck and Co., Inc., U.S.A., 1976, p. MISC-71.

the water of crystallization. Therefore, this result indicates that a water molecule is first adsorbed onto the dehydrated α-CaG crystal and is then incorporated into the crystal. Almost the same k-value was obtained from the peak at $2\theta=8.1°$ as from the peak at $2\theta=6.1°$ at any relative humidity, suggesting that the rate of rehydration is the same as that of growth of the dihydrate crystal, and that no intermediate crystalline state appears during rehydration.

For the CaG molecule, five crystal modifications due to differences in the amount of water of crystallization were observed. The main factor controlling the crystal modifications might be differences in the interaction between water and CaG molecules in the crystal. As such modifications were not observed in crystals of the sodium[3) and cadmium[4) compounds, they might be a result of a unique mode of interaction between water and calcium ions.