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X-ray Powder Diffraction by the Di-Sodium Salt of Adenosine Tri-Phosphate and its Hydrates

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The variation of the hydration of the di-sodium salt of adenosine tri-phosphate in atmospheres of varying humidity has been determined gravimetrically. Changes in the X-ray powder diffraction photograph have been related to changes in the degree of hydration of the compound. Three distinct X-ray powder patterns are described and their unit cells given.

Introduction

Adenosine tri-phosphate occurs naturally in muscle tissue and is a substance of considerable biochemical importance because of its function in storing and releasing the chemical energy of foodstuffs in a form suitable for utilization by living organisms. This function has been interpreted by Lipmann (1941) in terms of 'energy-rich' or 'high-energy' phosphate bonds which are imagined to exist in the molecule. It is therefore to be expected that the molecular structure of adenosine tri-phosphate will be of great interest crystallographically. Because of the complexity of the molecule it is perhaps not surprising that it is difficult to obtain crystals of the compound, but it has recently been shown by Berger (1956) that the di-sodium salt of adenosine tri-phosphate from muscle may be crystallized by adding an equal volume of ethyl alcohol to a 20% solution of the compound in water. This results in an oily precipitate from which small needle-shaped crystals separate after standing for about an hour at room temperature. Elementary analysis showed the composition of the crystals to be consistent with that required by the formula $Na_2H_{14}C_{10}N_5P_3O_{13}.3\,H_2O$. This compound is now obtainable commercially (from Sigma Chemical Company, St. Louis, Mo., U.S.A.) and has been used in the present study. Minor changes in the X-ray powder diffraction pattern of the compound were observed and were discovered to be due to changes in the degree of hydration of the compound, and this effect was studied.

Experimental

0·1 g. samples of the commercial compound were stored in atmospheres of constant humidity, maintained by aqueous solutions of sulphuric acid, until of constant weight. A small fraction of the sample was then removed and sealed between sheets of moisture-proof cellophane and its X-ray powder photograph was taken. The remainder of the sample was dehydrated by vacuum desiccation over phosphorus pentoxide and its water content was determined from the resulting loss in weight. In a second series of experiments 0·1 g. samples of the anhydrous compound were stored in atmospheres of constant humidity and the gain in weight was noted: the X-ray powder photographs of these rehydrated samples were also taken.

The powder photographs were taken in a 12·5 cm.-diameter camera using Soller-slit focusing. The dispersion in a camera of this type is the same as that in a conventional camera of 25 cm.-diameter. The lines on the photographs were slightly diffuse, presumably owing to the small crystalline size of the specimens, and the accuracy of measurement of reciprocal spacing was thus somewhat lower than that usually obtained with this camera. The estimated standard deviation in measuring a reciprocal spacing ($n/d = (2\sin\theta)/\lambda$) was ±0·0015 Å^{-1}, this figure being practically independent of θ in the range of spacings measured.

Results and discussion

The results of the gravimetric analysis are given in Fig. 1, which shows the degree of hydration of the com-

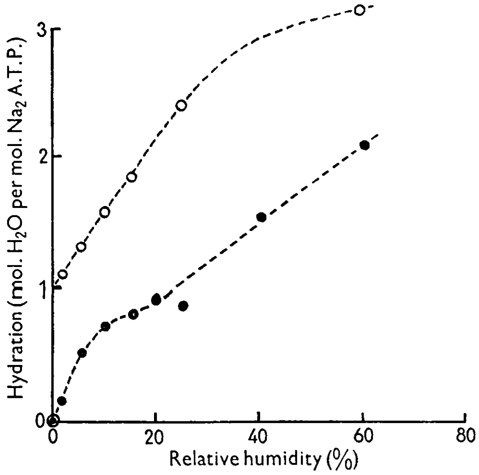

Fig. 1. Hydration of Na_2 A.T.P. plotted against relative humidity of ambient atmosphere. Dots: equilibrium water content of samples prepared by hydration of the anhydrous compound; circles: equilibrium water content of samples prepared by dehydration of crystals prepared by Berger's method.

Table 1. *Unit-cell dimensions of* Na_2 A.T.P.

Group	*a* (Å)	*b* (Å)	*c* (Å)	α (°)	β (°)	γ (°)
A	12·77	10·55	14·85	91·7	93·4	80·1
B	12·55	10·58	15·27	91·8	93·4	80·1
C	12·55	10·58	15·66	91·8	93·4	80·1

pound plotted against relative humidity of the ambient atmosphere. The upper curve shows how the water content of the commercial compound falls steadily as the relative humidity is decreased; at 0% relative humidity the compound is almost exactly the monohydrate, and this remaining water can be removed at room temperature only by vacuum desiccation over phosphorus pentoxide. The hydration curve for the anhydrous compound is always below that for the commercial compound, at least until the relative humidity is raised to very high values. When stored in atmospheres of 100% relative humidity, samples of the compound dissolve in absorbed water whatever their previous hydration history.

The X-ray photographs of all the crystalline samples examined fell into one of three distinct groups, the diffraction patterns of all samples in any group being identical. These patterns may for convenience be called *A*, *B* and *C*, and are best characterized by the value of the longest interplanar spacing observed on the diffraction photograph.

The existence of the three groups is displayed in Fig. 2, in which the value of longest spacing is plotted against the water content of the samples. To a very rough approximation the least hydrated specimens fall into group *A* with a spacing of 14·5±0·2 Å; and the most hydrated fall into group *C* with a spacing of 15·8±0·2 Å. The remaining specimens fall into the intermediate group *B* with a spacing of 15·2±0·2 Å. To some extent the X-ray pattern depends not only

Table 2. *Indexing, and comparison of observed and calculated spacings for powder photographs of adenosine tri-phosphate*

Index	Pattern *A*			Pattern *B*			Pattern *C*		
h k l	*I*	$(d/n)_o$	$(d/n)_c$	*I*	$(d/n)_o$	$(d/n)_c$	*I*	$(d/n)_o$	$(d/n)_c$
0 0 1	*vs*	14·5	14·6	*vs*	15·3	15·2	*vs*	15·7	15·6
1 0 0	—	—	—	—	—	—	*vw*	12·4	12·3
0 1 0	*s*	10·4	10·4	*w*	10·5	10·4	*w*	10·5	10·4
1 0 $\bar{1}$	—	—	—	*vw*	9·85	9·86	*vw*	9·90	9·95
1 0 1	—	—	—	—	—	—	*vw*	9·38	9·44
0 1 1	—	—	—	—	—	—	*vw*	8·63	8·67
0 0 2	*vw*	7·29	7·30	*m*	7·56	7·62	*s*	7·90	7·81
1 1 $\bar{1}$	*vw*	6·72	6·73	*w*	6·75	6·76	*w*	6·75	6·79
1 1 1	*vw*	6·52	6·48	*w*	6·54	6·51	*w*	6·58	6·54
0 1 $\bar{2}$	—	—	—	*s*	6·29	6·22	*md*	6·35	6·32
2 0 0	*vs*	6·27	6·28	—	—	—	—	6·19	6·17
2 $\bar{1}$ 0	—	—	—	*w*	5·77	5·76	*vs*	5·78	5·76
2 0 1	*w*	5·62	5·66	*m*	5·65	5·62	*w*	5·65	5·64
2 $\bar{1}$ $\bar{1}$	—	—	—	*w*	5·43	5·46	*vw*	5·44	5·48
1 1 $\bar{2}$	—	—	—	—	—	5·43	—	—	5·49
0 0 3	*vs*	4·89	4·87	*s*	5·15	5·08	*vs*	5·21	5·21
1 $\bar{2}$ 0	—	—	—	—	5·05	5·13	—	5·10	5·13
2 1 0	*m*	5·00	5·02	*s*	4·90	4·95	*m*	4·93	4·95
0 2 1	—	—	—	—	—	4·90	—	—	4·91
2 1 $\bar{1}$	—	—	—	*s*	4·68	4·77	*s*	4·76	4·79
2 1 1	*w*	4·68	4·66	—	—	4·63	—	4·64	4·65
$\bar{2}$ 2 0	—	—	—	*w*	4·44	4·37	*m*	4·39	4·37
1 2 $\bar{1}$	—	—	—	—	4·37	4·39	—	—	4·40
1 $\bar{2}$ 2	—	—	—	—	—	—	*m*	4·26	4·28
$\bar{2}$ 2 1	*w*	4·18	4·18	*s*	4·19	4·18	—	—	—
3 0 0	—	4·01	4·19	—	4·11	4·11	*m*	4·12	4·12
3 0 $\bar{1}$	—	—	4·08	—	—	—	*m*	4·02	4·03
3 0 1	—	—	3·97	—	—	—	—	—	—
1 2 2	*m*	3·81	3·79	—	—	—	—	—	—
0 0 4	—	3·67	3·65	*m*	3·806	3·810	*s*	3·902	3·906
3 0 $\bar{2}$	—	—	—	—	—	—	*w*	3·731	3·724
3 1 $\bar{1}$	—	—	—	—	—	—	*s*	3·546	3·570
0 3 0	*m*	3·466	3·465	*s*	3·462	3·474	*m*	3·470	3·474
0 3 $\bar{1}$	*w*	3·384	3·377	*vw*	3·401	3·406	*w*	3·401	3·407
3 0 $\bar{3}$	*m*	3·322	3·281	*m*	3·322	3·284	*m*	3·317	3·317
2 $\bar{3}$ 0	—	3·268	3·271	—	3·268	3·278	—	3·273	3·278
1 3 0	*s*	3·180	3·201	*m*	3·180	3·203	*m*	3·190	3·203
1 3 $\bar{1}$	—	3·130	3·152	—	3·130	3·157	—	—	—
1 3 1	—	—	3·110	—	—	3·115	—	—	—
4 0 0	—	—	—	—	—	—	*m*	3·105	3·086
4 0 $\bar{1}$	—	—	—	*vw*	3·053	3·057	—	3·058	3·058
—	*w*	3·021	—	*m*	2·994	—	*w*	2·937	—
—	—	2·976	—	*w*	2·869	—	*w*	2·850	—

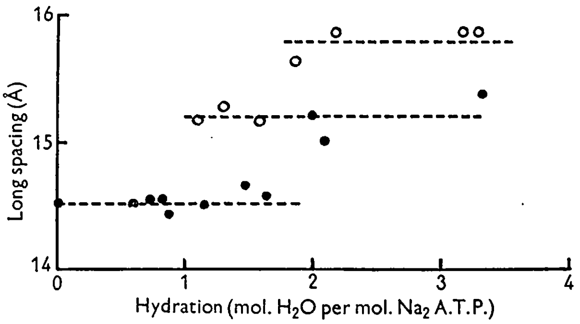

Fig. 2. Variation of the long spacing of Na_2 A.T.P. with hydration. Dots: spacing of samples prepared by hydration of anhydrous compound; circles: spacing of samples prepared by dehydration of crystals prepared by Berger's method.

on the degree of hydration of the sample but also on its previous history. For example it is possible to rehydrate an anhydrous sample almost to $1\frac{1}{2}$ H_2O and for the diffraction pattern to be in group *A*, while a previously highly hydrated sample may be dehydrated to 1·0 H_2O yet give the group-*B* diffraction pattern.

The higher orders, up to the fourth, of this longest spacing can be identified on the powder patterns since they, too, show corresponding movements from one group to another. There is also a set of haloes on the diffraction photographs which always appears in the same position irrespective of the group to which the pattern belongs. Thus if the longest spacing is indexed as 001 then these invariant spacings may be indexed as *hkl* with $l = 0$, or at least with l small, and it may then be concluded that the absorption of water into the crystal structure occurs in such a manner as to increase the *c* axis of the unit cell with relatively small changes in the other cell dimensions.

With the help provided by the movement of the 00*l* reflexions from group to group, and using Vand's (1948) graphical method, it was possible to index the powder photographs and to determine unit-cell dimensions for each of the three groups (Table 1).

The density of the commercial compound was determined in a helium densitometer similar to that described by Schumb & Rittner (1943). The apparatus was flushed many times with carefully dried helium so that it could be assumed that the moisture content of the sample was one molecule of water per molecule of Na_2 A.T.P. The observed value of the density (1·94±0·05 g.cm.$^{-3}$) agrees well with that calculated by assuming four molecules per unit cell (1·88±0·06 g.cm.$^{-3}$), and provides good confirmation that the X-ray photographs have been correctly indexed.

The reliability of the proposed unit cells may also be judged from Table 2, in which the indices of the observed lines are given and the calculated and observed values of the interplanar spacings are compared. In no case is there a significant discrepancy between the observed and calculated spacings and it is therefore concluded that the proposed cells are certainly possible interpretations of the powder photographs, and because of the agreement between the calculated and observed density and because prolonged searching produced no other alternative cells that they are indeed the true unit cells of the compound and its hydrates.

It is difficult to calculate the probable errors in the cell dimensions because of the ambiguity in indexing most of the shorter interplanar spacings, which is a consequence of the low symmetry and large volume of the cells. However, it is estimated that the probable error in the cell edges is less than 0·1 Å and that in the angles is less than 1°.

A fourth glassy modification of the compound also exists, and results when an aqueous solution is allowed to evaporate to dryness. This modification is quite transparent, optically inactive and very brittle. It gives a typical 'liquid' X-ray powder diffraction pattern with a single diffuse halo with maximum intensity at 4·3 Å.

References

Berger, L. (1956). *Biochim. Biophys. Acta*, **20**, 27.
Lipmann, F. (1941). *Adv. Enzymology*, **1**, 99.
Schumb, W. C. & Rittner, E. S. (1943). *J. Amer. Chem. Soc.* **65**, 1692.
Vand, V. (1948). *Acta Cryst.* **1**, 109.