CRYSTALLIZATION OF THE SODIUM SALT OF ADENOSINE TRIPHOSPHATE

by

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A method is described for the crystallization of the disodium salt of adenosine triphosphate (ATP)* by precipitation from aqueous solution with ethanol. The effect of variations of temperature, pH, and purity of the ATP are briefly discussed.

EXPERIMENTAL

Seven g of high purity amorphous sodium salt of ATP, isolated from muscle**, was dissolved in 40 ml water; the pH was 3.5. Upon addition of an equal volume of 95% ethanol at room temperature an oily precipitate formed which changed to crystals in about 10 minutes (Fig. 1). After standing at room temperature for one hour, the solution was clear and the crystals coated the bottom and sides of the glass vessel.

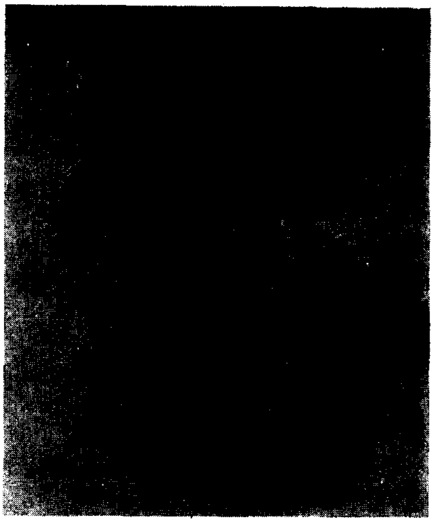

Fig. 1. Photomicrograph of crystals of disodium salt of ATP. Magnification = 400 diameters.

The crystals were collected by filtration and washed several times with absolute ethanol. The yield was 5.6 g after air-drying at room temperature overnight. An additional 0.7 g of crystals was obtained in a similar manner by adding 40 ml 95% ethanol to the filtrate.

The main crop of crystals was subjected to elemental analysis. As shown in Table I, the observed values agree favorably with theory for the disodium salt of ATP containing $3H_2O$; molecular weight = 605.

Inorganic P was determined by the method of FISKE AND SUBBAROW[1] on a freshly prepared solution of the crystals. Acid-labile P was estimated by hydrolysing for 7 minutes in N HCl at 100°. Total P was estimated after wet ashing with H_2SO_4 and

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* The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AtetraP, adenosine tetraphosphate. All temperatures are in degrees centigrade.

** Offered by Sigma Chemical Company as, "Chromatographed Grade" of ATP prior to the development of their crystalline preparation.

H_2O_2. Adenine content of the solution was calculated from the optical density at 260 mμ at pH 2 based on $E_{260} = 14{,}200$[2]. Except for the small amount of inorganic P, the observed values shown in Table II correspond to pure ATP. Except for a higher inorganic P content, the values reported in Table II also apply to the amorphous sodium salt of ATP from which the crystals were prepared.

TABLE I

ELEMENTAL ANALYSIS OF CRYSTALLINE ATP

Constituent	*Found** %	*Theory for $Na_2ATP \cdot 3H_2O$ (mol. wt. = 605) %*
C	19.64	19.85
H	3.39	3.31
N	12.04	11.58
P	15.61	15.38
Na	7.56	7.61
H_2O (loss on drying at 105°C)	8.59	8.92

* All determinations except Na were made by Elek Micro Analytical Laboratories of Los Angeles, California. Na was determined by flame photometry.

TABLE II

PHOSPHORUS CONTENT OF CRYSTALLINE ATP

	Found	*Theory of ATP*
Inorganic P as % of total P	0.05	0.00
Ratio of acid-labile to total P	0.666	0.666
Atoms of P per mole of adenine	3.00	3.00

While the elemental and phosphorus determinations reported in Tables I and II indicated that the crystals were essentially pure ATP, BOCK AND ALBERTY[3] have pointed out that these procedures are generally not sensitive enough to rule out the absence of AMP, ADP, or AtetraP. However, paper chromatography did establish the homogeneous nature of the crystals.

Two solvent systems were employed, both of which are capable of detecting 1 to 2% contamination by related adenosine compounds in ATP. One was *iso*butyric acid - conc. NH_4OH - H_2O (66:1:33) pH 3.8[4]. The other was the two-layer *iso*amyl alcohol--20% Na_2HPO_4 system[5].

The ascending boundary and ultraviolet detection methods of CARTER[6] were used. Only one spot was detectable on the paper after development for 18 hours in either system when crystalline ATP was applied. The amorphous sodium salt of ATP which was used to prepare the crystals was also free of contamination.

After storage of the crystals at room temperature for 3 months during the summer, AMP and ADP were detectable by paper chromatography. The inorganic P increased to 5% of the total P with a corresponding decrease in the acid-labile P. When stored at —20° for the same period of time, no significant amount of decomposition of the crystalline ATP was noted. This indicates that ATP decomposes at a rate of 2 to 5% per month at 28 to 33° with little or no decomposition at —20°. A single melting point determination was made on the crystals after storage at —20° for 8 days. The sample was placed in an oil bath which had been prewarmed to 110°, and the temperature was raised 3° per minute. The crystals melted with decomposition at 188° (corrected).

Effect of temperature

Addition of 2 volumes of 95% ethanol at 5° and —15° to a cold aqueous solution of the crystalline ATP at pH 3.5 resulted in the formation of oily precipitates which remained oily when stored overnight in an ice chest or deep freeze, respectively. However, when these same preparations were placed at room temperature, crystallization did occur after several hours. Crystallizations or recrystallizations were uniformly successful in about 30 minutes or less when the alcohol temperature was between 18° and 32°. Under otherwise similar conditions potassium ions could not replace sodium ions in the formation of crystalline ATP.

Effect of the purity of the ATP

Attempts to crystallize ATP from solutions known to contain significant amounts of other nucleotides were often successful but usually required a longer period of time; up to 2 hours. In other instances, seeding was required to induce crystallization. Generally, the crystalline ATP obtained from solution containing other nucleotides, was not free of these contaminants as evidenced by paper or ion exchange chromatography[2]. This demonstrates the need of a high purity ATP as the starting material if crystalline ATP of maximum purity is to be obtained; crystallization in itself does not necessarily produce maximum purity ATP.

Effect of pH

A solution of crystalline ATP (52 mg/ml) was adjusted from pH 3.5 to 7.5 with 7*N* NaOH. The pH was then readjusted to pH 0.5 by the dropwise addition of 6*N* HCl.

A 1 ml sample was removed at each pH indicated in Table III as the readjustment of pH was being made. Two volumes of 95% ethanol was added to each sample at room temperature and then observed grossly and microscopically for crystallization. Crystallization took place in 40 minutes or less in all samples of pH 4 or lower. No crystals formed at pH 5 or 6.5 even after standing overnight at room temperature.

TABLE III

EFFECT OF pH ON FORMATION OF CRYSTALLINE ATP (SEE TEXT)

pH	*Yield in mg*	*Nature of product*	*Atoms of Na/mole of adenine*
0.5	38	Crystalline	1.73
1.0	42	Crystalline	1.71
1.5	45	Crystalline	1.82
2.5	46	Crystalline	1.90
4.0	29	Crystalline	1.87
5.0	—	Amorphous	—
6.5	34	Amorphous	3.32

All precipitates were collected by centrifugation and washed twice with 5 ml portions of 67% ethanol, twice with 95% ethanol, and then dried in a vacuum desiccator for 30 minutes over alumina. The pH 5 sample was lost on centrifugation. Sodium and adenine were estimated on solutions of these products as previously described. It was assumed that these solutions were free of extraneous Na as NaCl because no chloride ion was detected when tested with $AgNO_3$ in dilute HNO_3. As shown in Table III, crystalline ATP containing approximately 2 atoms of Na per mole was formed at all pHs of 4 or lower.

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SUMMARY

Addition of 1 or 2 volumes of 95% ethanol at room temperature to aqueous solutions of the sodium salt of ATP at pH 4 or lower, resulted in the formation of the crystalline disodium salt of ATP.

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