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## 2-Azaadenosine triphosphate as a substitute for adenosine triphosphate in active transport of potassium across the erythrocyte membrane

It was reported previously that, when human erythrocytes preserved for more than 8 weeks were incubated with 2-azaadenine or 2,6-diaminopurine together with inosine, a considerable amount of the corresponding ATP analogue was accumulated within the cells[1]. These erythrocytes with unnatural nucleotides had a fairly high activity in the production of lactic acid from added inosine. Moreover, a preliminary experiment showed that both the ATP analogues could serve as substrates for yeast hexokinase. These findings stimulated us to investigate if the ATP analogues act as substitutes for ATP in active transport of cations across the erythrocyte membrane.

Long-stored human erythrocytes were pre-incubated in Krebs-Ringer phosphate solution containing inosine (10 mM) with addition of adenine or adenine analogue (2 mM) for 4 h at 37°, and then allowed to stand overnight at 4°. The cells were washed with $K^+$-free saline solution repeatedly in the cold and incubated in 3 vol. of Krebs–Ringer phosphate solution containing KCl (1–1.4 mM) and inosine (10 mM) for 5 h at 37°. Samples of the cell suspensions were taken out for analyses after various time intervals. The potassium concentration inside the cells was calculated from the values in the medium and whole suspension and the hematocrit. The sample taken after 3-h incubation was deproteinized with trichloroacetic acid and the extract subjected to chromatography on a Dowex-1 chloride column according to COHN AND CARTER[2] with slight modifications. The fractions containing ATP and its analogue were collected and the amount of each nucleotide was calculated from the absorbancies at 250 and 260 mμ.

Table I summarizes the results of a typical experiment. It can be seen that only 2-azaadenosine triphosphate and not the other ATP analogue was as effective as ATP for potassium uptake by the cells. The addition of ouabain to this system completely inhibited the activation produced by accumulation of 2-azaadenosine triphosphate as in the case of ATP regeneration.

As POST *et al.*[3] reported an ATPase in the erythrocyte membrane which required

TABLE I

UPTAKE OF $K^+$ BY ERYTHROCYTES WHICH HAD ACCUMULATED ATP AND ATP ANALOGUES

| *Added in preincubation* | *Ouabain* | *Nucleotides (μmoles/ml cells)* | | *Change in potassium (μmoles/ml cells/h)* |
|---|---|---|---|---|
| | | *ATP* | *ATP analogue* | |
| None | – | 0.12 | — | 0.00 |
| 0.2 mM adenine* | – | 0.54 | — | 0.54 |
| Adenine | – | 1.34 | — | 0.86 |
| | + | 1.36 | — | −0.12 |
| 2,6-Diaminopurine | – | 0.13 | 0.52 | 0.08 |
| | + | 0.14 | 0.53 | 0.06 |
| 2-Azaadenine | – | 0.13 | 0.70 | 0.60 |
| | + | 0.13 | 0.69 | 0.00 |

* The concentration of adenine was reduced to 0.2 mM.

TABLE II

ACTION OF ERYTHROCYTE-MEMBRANE ATPASE ON ATP AND ATP ANALOGUES

Complete assay system was the same as employed by POST *et al.*[3]. The concentration of sodium was 80 mM and of potassium 32 mM. The activity is expressed as μmoles $P_i$ liberated during 1-h incubation at 40° per mg dry wt. of the enzyme. Ouabain was added in final concentration of $8 \cdot 10^{-5}$ M.

| *Substrate* | *Complete* | *Without $Na^+$ and $K^+$* | *With addition of ouabain* |
|---|---|---|---|
| ATP | 0.96 | 0.59 | 0.55 |
| 2,6-Diaminopurine riboside triphosphate | 0.14 | 0.18 | 0.14 |
| ATP | 2.06 | 1.28 | 1.26 |
| 2-Azaadenosine triphosphate | 1.86 | 1.20 | 1.27 |

both sodium and potassium and was inhibited by ouabain the enzyme activity with the ATP analogues as substrate was investigated. As seen in the Table II, 2-aza-adenosine triphosphate was hydrolysed as rapidly as ATP in the presence of membrane ATPase and the activity was dependent upon $Na^+$ and $K^+$ and inhibited by ouabain. The enzyme action on 2,6-diaminopurine riboside triphosphate was much less, and a dependency on $Na^+$ and $K^+$ and an ouabain sensitivity were not seen.

These findings offer further evidence for the view that membrane ATPase is a part of a system for active transport of cations in erythrocyte.

It has been observed that when the ATP level in the aged erythrocytes is restored by incubation with both adenine and inosine, the shape of the cells changes from a smooth sphere to a shallow-bowl form[4]. The same transformation took place when 2-azaadenosine triphosphate, but not 2,6-diaminopurine riboside triphosphate, was accumulated in the cells. It is suggested that the principal functions of the erythrocyte membrane, active transport of cations and maintainance of the characteristic shape, are determined by a common structural element involving ATPase activity.

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[1] M. TATIBANA AND H. YOSHIKAWA, *Biochim. Biophys. Acta*, 57 (1962) 613.
[2] W. E. COHN AND C. E. CARTER, *J. Am. Chem. Soc.*, 72 (1950) 4273.
[3] R. L. POST, C. R. MERRIT, C. R. KINSOLVING AND C. D. ALBRIGHT, *J. Biol. Chem.*, 235 (1960) 1796.
[4] M. NAKAO, T. NAKAO, M. TATIBANA AND H. YOSHIKAWA, *J. Biochem. Tokyo*, 47 (1960) 661.

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## Interference by menadiol in the colorimetric estimation of nitrite

During the course of our investigations on the properties of a menadione-dependent nitrate reductase in *Agrobacterium tumefaciens* (unpublished work) we observed that the enzyme activity as measured by nitrite formation was drastically decreased as