Specific rotations were measured in a 1 dcm tube.

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Effect of Adenosine Triphosphate and Monovalent Cations on Brain 5'-Adenylic Acid Deaminase

It was recently reported that the adenylic acid deaminase from human erythrocytes required ammonia or a monovalent metal ion as an essential co-factor[1]. It has also been reported that ATP activates this enzyme from dog brain[2] and beef brain[3]. In this report the effect of the simultaneous presence of ATP and a monovalent cation on the rabbit brain adenylic acid deaminase is presented.

Partially purified enzyme, in a soluble form, was prepared from rabbit brain acetone powder according to Mendicino and Muntz[2]. The enzyme solution obtained from the last sodium sulphate precipitation was dialysed against 400 volumes of 5 mM *tris* (*p*H 7·1), at 4° for 25 h. This preparation was quite unstable and lost all activity after 48 h. A more crude preparation with improved stability was obtained as follows : brain was homogenized with seven volumes of 0·25 M sucrose in a glass homogenizer with a 'Teflon' plunger. The homogenate was centrifuged at 18,000*g* for 45 min. The supernatant from this step was dialysed against 400 volumes of 5 mM *tris* (*p*H 7·1), at 4° for 24 h. The cloudy solution was centrifuged at 80,000*g* for 45 min. The clear supernatant was used as the source of enzyme. It retained its full activity after 2 weeks of storage at 4°. The adenylic acid deaminase activity of a 1 : 5 dilution of this preparation and that of the partially purified preparation under various conditions are presented in Table 1.

Table 1. Effect of Na^+ and Nucleotides on Brain Adenylic Acid Deaminase

Enzyme	2 mM AMP	50 mM Na^+	Nucleotides	$-\Delta A_{265}$
	+	−	—	0
	+	+	—	0
	+	−	2 mM ATP	0·070
'Crude'	+	+	2 mM ATP	0·110
	−	+	2 mM ATP	0
	+	−	0·2 mM ATP	0
	+	+	0·2 mM ATP	0·800
	+	+	2 mM ADP	0
	+	+	2 mM ITP	0
	+	−	—	0
'Purified'	+	+	—	0
	+	−	2 mM ATP	0·021
	+	+	2 mM ATP	0·127

The system contained 5 μmoles of AMP, 100 μmoles of *tris*-HCl (*p*H 7·1), the indicated amounts of *tris* salts of nucleotides and NaCl, and 0·5 ml. of the crude or the partially purified enzyme. Total volume was 2·5 ml. The reaction mixtures were incubated at 37° for 0·5 h, then deproteinized by the addition of 1·5 ml. of 8 per cent $HClO_4$. Absorbancy at 265mμ was measured on 1:30 dilution of the deproteinized reaction mixtures and controls which did not contain any enzyme.

Deaminase activity was determined according to Kalckar[4] using a Gilford spectrophotometer. The rate of deamination was a linear function of time during the disappearance of the first 25 per cent of the substrate. Inorganic phosphate was determined according to Fiske and Subbarow[5].

The results in Table 1 show that the enzyme, at the concentration used, may be activated by ATP alone but not sodium ion alone. The synergistic effect of ATP and the cation is best observed at low ATP concentrations. When neither ATP nor the cation has any effect, the simultaneous presence of both activates the enzyme. The enzyme does not deaminate ATP or ADP. The activating effect of ATP is also quite specific. Neither ADP nor ITP activates the deamination of AMP. It should also be noted that the particle-free homogenate as described here is quite similar to the partially purified deaminase from acetone powder in respect to other properties[2]. In the absence of Mg^{++} and at *p*H 7·1, the 1 : 5 dilution of the crude enzyme did not release any detectable inorganic phosphate from either ATP or AMP. That all the monovalent cations do not have similar activating effect on the enzyme is evident from the data presented in Table 2. Of the cations tested sodium was the best activator.

Table 2. Effect of Various Cations on Brain Adenylic Acid Deaminase

	Na^+	K^+	Rb^+	Cs^+	NH_4^+	$N(CH_3)_4^+$	Ca^{++}	Mg^{++}
$-\Delta A_{265}$	0·076	0·036	0·030	0·00	0·050	0·00	0·00	0·00

The reaction mixture contained 5 μmoles of AMP, 0·5 μmoles of *tris*-ATP, 100 μmoles of *tris*-HCl (*p*H 7·1), 125 μmoles of the chloride salts of the indicated ions, and 0·5 ml. of the 'crude' enzyme. Other conditions the same as in Table 1.

Mendicino and Muntz[2] concluded that the activating effect of ATP on brain adenylic acid deaminase was not likely due to a chemical reaction of ATP either through the phosphate or the amino groups ; they proposed a loose binding between ATP and the enzyme. Experiments described here suggest that the presence of both ATP and a monovalent cation are essential for the active form of the enzyme. Obviously the nature of the interactions involved has yet to be resolved. However, it should be noted that Ling[6] on theoretical grounds has suggested that ATP binding to proteins may play an essential part in the electrostatic binding of monovalent metal ions to anionic sites of proteins. According to this hypothesis a quantitative relationship between the amount of bound ATP and the selectively adsorbed monovalent metal ions by the proteins should exist. If the activating effects of the cations on the adenylic acid deaminase indeed prove to be due to their direct binding on the enzyme protein, this may be an experimental support of Ling's hypothesis on the role of ATP as a "cardinal anion".

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Identification of O-Aminoacetophenone as a Flavour Compound in Stale Dry Milk

In our efforts to improve the keeping quality of dry milk products, attention has been directed towards isolating and identifying flavour compounds present in stale products. This communication deals with the isolation of a compound with a 'grape-like' flavour, identified as *o*-aminoacetophenone. Preliminary work on the solvent extraction of stale non-fat dry milk indicated that a strong flavour fraction characterized as potato-like or cocoa-like could be extracted with hexane. The odour was materially altered by adding a strong acid but could be regenerated by adding alkali. Crude concentrates were prepared by hydrochloric acid extraction of hexane extracts of stale dry milk powder. The alkali-liberated flavour fraction was back extracted in small volumes of hexane which