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Ammonia Formation in Brain

2. BRAIN ADENYLIC DEAMINASE

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During our studies on the ammonia formation of brain slices (Weil-Malherbe & Green, 1955) the possibility was envisaged that adenylic deaminase might be the terminal enzyme, acting perhaps as a component of a reamination-deamination cycle. The study of this enzyme was originally undertaken in the hope that it might yield evidence bearing on this hypothesis.

Muntz (1953) recently described an unusual type of adenylic deaminase in aqueous extracts from acetone-dried dog brain. The enzyme was strongly activated by adenosine triphosphate, an effect that was greatest when the concentrations of adenosine triphosphate and adenylic acid were equal. The ammonia formation from adenylic acid alone was interpreted as being secondary to the conversion of adenylic acid into adenosine.

In the present paper observations on the purification of this enzyme and on the properties of the purified preparation are reported.

EXPERIMENTAL

Methods

Abbreviations. Adenosine triphosphate, adenosine diphosphate and 5'-adenylic acid are denoted by ATP, ADP and AMP. ITP, IDP and IMP stand for the corresponding

inosine phosphates. Ethylenediaminetetraacetate is denoted EDTA and trichloroacetic acid TCA.

Analytical. Enzyme activity was measured either by the estimation of ammonia or spectrophotometrically. Filtrates deproteinized with TCA at a final concentration of 5% (w/v) were used for the ammonia estimations, which were carried out by the microdiffusion method (Conway, 1950) and nesslerization; in some experiments the filtrates were analysed by direct nesslerization. The spectrophotometric method is based on the decrease of extinction at 265 m μ . and the increase at 240 m μ . when adenine nucleotides are deaminated to hypoxanthine nucleotides. In a solution with a nucleotide content corresponding to 1 μ g. adenine/ml. $\Delta E_{265 \text{ m}\mu} = -0.059$ and $\Delta E_{240 \text{ m}\mu} = +0.032$ (Kalckar, 1947). Since the enzyme preparations were turbid, the solutions were deproteinized with HClO₄ at a final concentration of 3% (w/v). The filtrates were diluted to contain $0.3\text{--}1 \times 10^{-4} \text{M}$ total nucleotides. A Unicam SP. 500 quartz spectrophotometer was used for the measurements.

Phosphate was estimated by a modification of the method of Martin & Doty (Weil-Malherbe & Green, 1951; Weil-Malherbe, 1953) and protein by the method of Lowry, Rosebrough, Farr & Randall (1951).

Standard conditions. For activity measurements the enzyme was incubated in a solution containing 0.0033M AMP, 0.0033M ATP, 0.02M sodium succinate buffer (pH 6.2) and 0.01M sodium EDTA (pH 6.8), in a total volume of 1.5 ml. or multiples thereof. The final pH of the

mixture was about 6.7. The buffering capacity of succinate at this pH is slight, but enzyme activity was higher in this mixture than in other buffers (see below). Incubation was carried out in stoppered test tubes at 30° for 30 or 60 min.

Other experimental procedures are described in the following section.

Materials. ATP was prepared by the method of Dounce, Rothstein, Beyer, Meier & Freer (1948) and purified by ion-exchange chromatography (Cohn & Carter, 1950). ADP was prepared by incubating ATP with glucose and purified yeast hexokinase (fraction 3a of Berger, Slein, Colowick & Cori, 1946). For ITP and creatine phosphate see Weil-Malherbe & Green (1955). IDP was prepared from ADP by treatment with HNO_3 . (Found: total organic P, 8.1; acid-labile P, 4.31; inorganic P, 0.15%; N, 7.81; pentose 20.0%; molecular extinction coefficient at 250 $\text{m}\mu$, 12400. Calc. for $\text{C}_{10}\text{H}_{11}\text{O}_{11}\text{N}_4\text{P}_2\text{Ba}_{1.5}\cdot 6\text{H}_2\text{O}$: total P, 8.39; acid-labile P, 4.20; N, 7.59; pentose, 20.3%.)

AMP was obtained from Zellstoff-fabrik Waldhof, Wiesbaden, Germany, IMP from Boots Pure Drug Company, Nottingham.

Results

Purification. The purification of brain adenylic deaminase turned out to be more difficult than anticipated, mainly on account of the instability of the enzyme. It loses activity after some days, even in the cold, and especially after partial purification. There were, however, exceptions and some fractions remained active for 2-3 weeks.

After centrifuging, almost all of the activity remained in the supernatant. It was found, however, that the activity was still associated with particulate matter which could be spun down completely at 15 000 g. The high-speed centrifugate contained only traces of protein (<0.1 mg./ml.). A freshly prepared high-speed residue, suspended in 0.1M succinate buffer (pH 6.2) (one-third of the volume before high-speed centrifuging), was used for most experiments. This fraction, which is henceforth referred to as the 'particulate fraction', could be freeze-dried and, though some activity was lost, the resulting material was comparatively stable.

In the following the details of an actual preparation are given. 40 g. acetone powder were stirred in 400 ml. ice-cold glass-distilled water for 15 min. The mixture was centrifuged in an angle centrifuge (MSE Angle Head 6886, Measuring and Scientific Equipment Ltd., London) for 30 min. at 4000 rev./min. and 3°. The supernatant was again centrifuged for a further period of 2 hr. The slightly turbid solution (220 ml.) was mixed with 94 ml. saturated $(\text{NH}_4)_2\text{SO}_4$ solution. After centrifuging, the precipitate was dissolved in a small volume of water. The resulting solution was 8% saturated with respect to $(\text{NH}_4)_2\text{SO}_4$. Saturation was increased to 40% by the addition of solid $(\text{NH}_4)_2\text{SO}_4$ (19 g./100 ml.). The precipitate was centrifuged off, dissolved in glass-distilled water and dialysed at 3° against 10 l. running glass-distilled water over a period of 48 hr. The dialysed solution (130 ml.; pH 6.40) was adjusted to pH 7.0. A portion of 20 ml. was mixed with 8 ml. alumina gel C γ (320 mg. dry wt./10 ml.) and shaken for 5 min. The

Table 1. *Preparation of purified adenylic deaminase from 40 g. of acetone-dried powder of ox brain*

The activity tests were carried out under standard conditions for 30 min.

Fraction	Protein content of fraction (mg.)	Activity ($\mu\text{g. NH}_3/\text{mg. protein}$)
Aqueous extract of powder	1660	7.8
After $(\text{NH}_4)_2\text{SO}_4$ fractionation	415	31.4
After treatment with alumina gel C γ	79	133

Table 2. *Adenylic deaminase activity of extracts of brain acetone powders prepared from grey and white matter separately*

Nucleotides added (0.0033M)	$\mu\text{g. NH}_3/5 \text{ mg. protein/hr.}$	
	Grey matter	White matter
AMP	13.9	8.4
ATP	2.8	3.5
AMP + ATP	67.6	72

Since the activity tended to deteriorate, a simple and rapid procedure had to be adopted. It consisted essentially of two steps: a fractionation with ammonium sulphate and a treatment with aluminium hydroxide gel C γ (Willstätter & Kraut, 1923, 1924). Acetone-dried ox brain was the starting material. The aqueous extract was brought to 30% saturation with $(\text{NH}_4)_2\text{SO}_4$. The precipitate was dissolved and reprecipitated by $(\text{NH}_4)_2\text{SO}_4$ at 40% saturation. The fraction was thoroughly dialysed against running distilled water and was then shaken with sufficient alumina C γ at pH 7.0 to adsorb about 80% of the protein

gel was removed by a short centrifuging at 1500 g. The supernatant was now centrifuged for 15 min. in the MSE Superspeed unit at 15 000 g and the residue suspended in 7 ml. succinate buffer.

The purification factor attained in this preparation was 16.9 (Table 1). It varied in other experiments between 10.2 and 21.3. 81% of the original activity was recovered.

Crude aqueous extracts had the same activity whether they were prepared from the acetone-dried grey or white matter of ox brain (Table 2). The whole brain was therefore used in the preparation of the acetone powder.

Solvent fractionation. The aqueous extract of brain acetone powder could also be fractionated by acetone or ethanol precipitation, but only with considerable losses of activity. The highest activity was found in the fractions precipitated between 20 and 30% ethanol (v/v) and between 30 and 40% acetone (v/v), but in both cases only about 25% of the original activity were recovered.

Solubilization. Attempts were made to solubilize the enzyme by treatment with *n*-butanol, according to Morton (1950). The solvent was stirred with 3 vol. enzyme solution either at 30° or at 0° and the activity of the aqueous layer

tested after dialysis. The enzyme solutions thus treated were water or buffer extracts of acetone powder or fractions precipitated by $(\text{NH}_4)_2\text{SO}_4$. In every case the enzyme was found to have been inactivated. In another experiment a freeze-dried particulate fraction was suspended in *n*-butanol at -20° . The mixture was centrifuged at -10° and the residue dried at -10° *in vacuo* (0.1 mm. Hg). The dry powder was extracted with 0.1M succinate buffer and centrifuged at 15 000 *g*. Though the precipitate was still active, no activity had passed into solution.

In a further series of experiments the particulate fraction, in samples of 0.5–1 mg. protein, was suspended in 1.5 ml. of a solution containing buffer and a bile salt and stirred for 15 min. at room temperature. After centrifuging at 15 000 *g* for 15 min. in the cold, the supernatant and insoluble fractions were tested separately, the latter after resuspension in the original volume of buffer solution. A suspension, treated with bile salt, but not centrifuged, and another, not treated with bile salt, served as controls.

Sodium deoxycholate was used at concentrations of 3, 1, and 0.33% (w/v). It was highly inhibitory at all levels. No activity was found in any of the supernatant fractions and only with the lowest concentration was any activity found in the insoluble fraction. Results were similar with sodium tauroglycocholate. With sodium cholate, on the other hand, some solubilization apparently occurred, as shown by the appearance of activity in the supernatant fractions (Table 3). The inhibitory effect of the bile salt is obvious from its effect on the whole suspension. In the absence of the bile salt the sum of the activities of the soluble and insoluble fractions accounted for that of the whole suspension, but in its presence the activities of the centrifuged fractions exceeded the activity of the whole suspension, a discrepancy that increased with increasing bile salt concentration. This is due to the fact that the insoluble fraction was resuspended in buffer solution free from inhibitor.

Presence of other enzymes in the particulate fraction. As shown in Table 4, the particulate fraction possesses some

Table 3. *Solubilization of adenylic deaminase by sodium cholate*

Sodium cholate concn. (g./100 ml.)	Protein (mg.)	Activity of fractions ($\mu\text{g. NH}_3/50 \text{ min.}$)		
		Not centrifuged	Supernatant	Insoluble (resuspended)
0	0.16	53.0	1.8	49.5
0.25	0.16	35.8	16.8	26.8
0.5	0.16	26.8	16.8	18.3
1.0	0.21	10.8	7.3	15.0

Table 4. *Enzyme activities of particulate fraction*

Medium A: 0.06M sodium glycylglycine buffer (pH 7.1), 0.005M-MgCl₂, 0.005M-KCl. Medium B: 0.05M sodium succinate buffer (pH 6.2), 0.01M EDTA, 0.005M-KCl. Concentration of substrates: 5 $\mu\text{moles}/1.5 \text{ ml.}$ (0.0033M).

Substrates added	Formation of inorganic phosphate		Formation of NH_3 Medium B
	Medium A	Medium B	
	(μmoles/mg. protein/hr.)		
Adenosine	0	0	0.14
AMP	1.03	0.13	1.20
ATP	2.33	0.40	0.25
AMP + ATP	1.70	0.50	6.50

5-nucleotidase and ATPase activity in the presence of Mg^{2+} , though very little in its absence. Adenosine deaminase activity is also very slight. The ammonia formation found when AMP is the only substrate, therefore, cannot be accounted for by adenosine deaminase activity, as Muntz had assumed; moreover, the ammonia formation from AMP in the Mg-free medium was 9 times higher than the formation of inorganic phosphate. The particulate fraction also had weak myokinase activity, but this was apparent only in a medium containing glutathione and histidine (Table 5). Under standard conditions myokinase activity was practically absent.

The activating effect of ATP. The effect of an equimolar amount of ATP on the deamination of 5 μmoles AMP is illustrated in Fig. 1. In this experiment about 20 min. were required for 50% deamination in the presence of ATP, as compared with 3.3 hr. in its absence. In the absence of ATP the deaminating activity varied, but never disappeared completely (see also Tables 4 and 7); as it was unaffected by repeated washing or by dialysis of the particulate fraction, it is unlikely that it can be attributed to traces of ATP in the preparation. On the other hand, fractionation of the crude brain extract yielded preparations in which the activation effect of ATP was slight or lacking, without nucleoside being formed at a sufficient rate to implicate adenosine deaminase (Table 6). It may be assumed therefore either that there are two different adenylic deaminases, one responsive to the activating effect of ATP and the other not, or that the effect of ATP requires an additional factor that was absent or inactive in some fractions. By contrast, the treatment with alumina gel greatly enhanced the activating effect of ATP while leaving the activity without ATP unchanged.

Table 5. *Myokinase activity of particulate fraction*

Medium A: 0.01M sodium succinate buffer, 0.005M glucose, 0.067% (w/v) glutathione, 0.0067M histidine, 0.0067M-MgCl₂, 10 $\mu\text{g.}$ yeast hexokinase/ml. Medium B: 0.033M sodium succinate buffer, 0.005M glucose, 0.0067M-MgCl₂, 10 $\mu\text{g.}$ yeast hexokinase/ml. Medium C: as medium B, but with 0.01M EDTA instead of MgCl₂. All tests contained 5 μmoles ADP in 3 ml. (0.00167M).

	Δ Acid-labile phosphate	Δ Glucose
	(μmoles/mg. protein/30 min.)	
Medium A	-0.75	-0.8
Medium A, without yeast hexokinase	0	-0.2
Medium B	-0.21	0
Medium C	-0.16	0

Table 6. *Effect of enzyme fractionation on the formation of ammonia and inorganic phosphate*

Standard conditions. Substrates at 0.0033M concentration.

In presence of	...	...	NH ₃ formation			Phosphate formation
			AMP + ATP	AMP	Adenosine	AMP
Fractions			(μmoles/mg. protein)			
(NH ₄) ₂ SO ₄	20-30% sat.		2.94	0.52	0.55	0.12
	30-35% sat.		0.54	0.34	0.62	0.12
	40-50% sat.		0.32	0.32	1.52	0.12
Ethanol	20-30% (v/v)		1.70	0.53	0.55	0.15
	30-40% (v/v)		0.41	0.50	0.41	0.14
(NH ₄) ₂ SO ₄	0-40% sat.		1.26	0.63	0.36	—
Particulate fraction			6.65	0.64	0	—

The effect of ATP is specific. Replacement of ATP by ADP, ITP or IDP only led to insignificant activation (Table 7). This fact is demonstrated much more clearly with the purified enzyme than with the preparations used by Muntz. In a crude dialysed aqueous extract of brain acetone powder ADP had the same activating effect as ATP; it was only slightly decreased in a Mg-free medium where myokinase was strongly inhibited, as shown by the inhibition of the ammonia formation from ADP (Table 8). Creatine phosphate, in the presence of Mg²⁺, inhibits the

Fig. 1. Time curve of ammonia formation by particulate adenylic deaminase fraction. Nucleotides: 5 μmoles/1.5 ml.

Table 7. *Activation of adenylic deaminase by various nucleotides*

0.4 mg. protein (particulate fraction)/1.5 ml. Concentration of nucleotides: 0.0033M.

Addition	NH ₃ (μg.)
AMP	3.0
AMP + ATP	42.6
AMP + ADP	6.6
AMP + ITP	5.4
AMP + IDP	4.5

Fig. 2. Effect of ATP at varying concentrations of AMP on adenylic deaminase activity. Activity measured by ammonia formation in first 10 min.

Table 8. *Effect of Mg²⁺ and EDTA on ammonia formation by dialysed aqueous extract of brain acetone powder (1 in 10)*

0.3 ml. extract/1.5 ml., 30 min.

Additions (0.0033M)	With 0.005M- MgCl ₂	With 0.01M EDTA	Effect of absence of Mg ²⁺
	(μmoles NH ₃)		
ATP	1.0	0.13	Inhibition of ATPase
ADP	1.22	0.18	Inhibition of myokinase
AMP	1.5	1.28	—
ATP + AMP	4.6	4.6	—
ADP + AMP	4.6	4.12	—
AMP + creatine phosphate	0.52	1.2	Inhibition of creatine phosphokinase and/or myokinase
ATP + AMP + creatine phosphate	0.9	4.6	

deamination of AMP and its activation by ATP, no doubt owing to competition of creatine phosphokinase. The effect is suppressed if Mg^{2+} is absent.

According to Muntz the highest activation is obtained when ATP and AMP are present in equal concentration. At a low concentration of AMP maximum activation should therefore be observed at lower ATP concentrations than at a high concentration of AMP. However, when care is taken to measure the reaction rate during the near-linear phase, activity/concentration curves of ATP are obtained that are of a similar shape at different levels of AMP concentration, reaching a maximum between 3 and 5 mM ATP (Fig. 2).

pH Optimum. The pH/activity curve of the enzyme has a broad plateau between pH 6.2 and 6.8, when measured in pyrophosphate buffer, both with and without ATP (Fig. 3). This is a little more towards the acid side than the optimum found by Muntz.

Fig. 3. Effect of pH on activity of adenylic deaminase in 0.02M sodium pyrophosphate buffers.

Effect of cations and anions. Replacement of Na^+ by K^+ did not affect the activity of the enzyme. On the other hand, the multivalent cations that were tested inhibited it, with the exception of Ca^{2+} (Table 9). The addition of EDTA was, of course, omitted in these experiments.

Among anions, phosphate was found to have a marked inhibitory effect, in contrast to arsenate which inhibited only slightly. Pyrophosphate, too, caused some inhibition (Table 10).

Other inhibitors. The enzyme was inhibited by fluoride, *p*-chloromercuribenzoate and octan-2-ol (Table 11), but not by the following substances: arsenite, azide, cyanide, hydroxylamine, iodoacetate, 2:4-dinitrophenol, sulphanilamide (all at $10^{-3}M$), DL-methionine sulfoxide (mixed diastereoisomers), benzaldehyde, ferrocyanide and urethane (at $10^{-2}M$). Neither is the reaction inhibited by the end products, IMP or ammonia, at 0.01M concentration, in the presence or absence of ATP.

End products. It is evident from Fig. 1 that the ammonia formation quantitatively accounts for the deamination of one of the two compounds present, AMP or ATP. Very little inorganic phosphate is formed under standard conditions (Table 4). Analysis of the final reaction mixture by paper chromatography and paper electrophoresis has

shown that AMP is converted into IMP, while ATP is recovered unchanged.

It was found that paper chromatography with a solvent containing ethanol (75 vol.) and M ammonium acetate (pH 7.5) (30 vol.) (Paladini & Leloir, 1952) brought about a good separation of ATP (R_F 0.37) and AMP (R_F 0.61), but not of AMP and IMP. A solvent containing 100 ml. *iso*-butyric acid, 60 ml. *N-NH*₃ and 1.6 ml. 0.1M EDTA (Krebs & Hems, 1953), on the other hand, satisfactorily separated IMP (R_F 0.38) and AMP (R_F 0.64), but not ATP and IMP.

Table 9. Inhibition of adenylic deaminase activity of particulate fraction by cations

Standard conditions.		
Additions	Concentration (M)	Inhibition (%)
$CaCl_2$	0.01	0
$MgCl_2$	0.01	21
$MnSO_4$	0.01	24
$CoSO_4$	0.002	50
$CuSO_4$	0.002	67
$ZnSO_4$	0.002	57
$FeCl_3$	0.002	48

Table 10. Relative activity of adenylic deaminase of particulate fraction in various buffer solutions (0.02M, pH 6.69 ± 0.05)

Sodium succinate	100
Sodium borate-succinate	91
Sodium acetate-veronal	82
Sodium arsenate	82
Sodium pyrophosphate	70
Sodium phosphate	52

Table 11. Inhibition of the adenylic deaminase activity of particulate fraction

	Concentration (M)	Inhibition (%)
NaF	0.01	71
<i>p</i> -Chloromercuribenzoate	0.001	65
Octan-2-ol	Saturated soln.	42

A method was therefore devised in which a first run was carried out with the ethanol-ammonium acetate solvent in the descending direction for 18 hr. The spots were located by the photoprint method (Markham & Smith, 1949, 1951) and the paper strip was cut across between the ATP spot and the confluent spots corresponding to AMP and IMP. The distal part of the paper carrying the AMP and IMP spots was now subjected to a second run with ammonium *isobutyrate* solvent in the ascending direction for 20 hr. The spots, which by now were well separated, were again located by the photoprint method. The absorbing areas, including that cut off after the first run, were cut out and eluted twice with 5 ml. water. The extinction of the eluates was measured at 250 and 260 $m\mu$. and the concentration of the nucleotides was calculated, a molecular extinction coefficient of 15 800 at 260 $m\mu$. being assumed for AMP and ATP (Kalckar, 1947) and one of 12 300 at 250 $m\mu$. for

IMP (Schulman & Buchanan, 1952). The ratio E_{250}/E_{260} which is 1.60 for IMP and 0.83 for AMP and ATP was determined as an indicator of identity.

Samples of 1 ml. were withdrawn from the standard reaction mixture at the beginning and the end of the incubation and delivered into 1 ml. 10% (w/v) TCA. The filtrate was extracted 4 times with ether, the ether removed *in vacuo* and the volume made up to 2 ml. Samples of 0.3 ml., in portions of about 5 μ l., were applied to the paper (Whatman no. 1, 48 $\times$ 8 cm., pretreated according to Eggleston & Hems, 1952). Neglecting the fraction of the solution lost during the filtration the amount of each of the two nucleotides applied to the paper strip was 0.5 μ mole.

On the paper strip carrying the initial sample, two spots were located which occupied positions corresponding to

DISCUSSION

The results obtained with a purified enzyme preparation on the whole confirm the conclusions reached by Muntz (1953) on the basis of experiments with crude extracts. They show that brain contains an adenylic deaminase which is strongly activated by ATP. However, ATP does not appear to be an indispensable cofactor, since the enzyme possessed some activity even in the complete absence of ATP. No indication was found for a stoichiometric requirement for ATP; the mechanism by which ATP accelerates the reaction is obscure, as it is recovered unchanged. It is

Table 12. *Paper chromatography of reaction mixture before and after incubation with particulate fraction for 2 hr.*

Sample	Position of spot	$E_{250 \text{ m}\mu}$	$E_{260 \text{ m}\mu}$	$E_{250 \text{ m}\mu}/E_{260 \text{ m}\mu}$	$\mu\text{moles/spot}$
Initial	ATP	0.500	0.605	0.83	0.38
	AMP	0.590	0.695	0.85	0.44
Final	ATP	0.490	0.590	0.83	0.37
	IMP	0.525	0.337	1.56	0.43

Table 13. *Electrophoresis on paper of reaction mixture before and after incubation with particulate fraction for 2 hr.*

Sample	Position of spot		$E_{250 \text{ m}\mu}$	$E_{260 \text{ m}\mu}$	$E_{250 \text{ m}\mu}/E_{260 \text{ m}\mu}$	$\mu\text{moles/spot}$
	Distance travelled (cm.*)	Reference compound, having the same rate of movement				
Initial	20.5	ATP	0.645	0.755	0.85	0.48
	6.0	AMP	0.650	0.760	0.85	0.48
Final	20.0	ATP	0.605	0.710	0.85	0.45
	13.0	IMP	0.560	0.380	1.47	0.45

* Measured from centre of spot.

ATP and AMP; on the strip carrying the final sample again only two spots were found in the positions of ATP and IMP, while there was no absorption corresponding to AMP. The results of the spectrophotometric analysis of the eluates are shown in Table 12. They confirm that ATP was unchanged, whereas AMP was quantitatively converted into IMP.

Identical results were obtained by the method of paper electrophoresis. The EEL paper-electrophoresis apparatus (Evans Electroselenium Ltd., Harlow, Essex), which was slightly modified so as to take a single paper strip of 35 $\times$ 15 cm., was used, with 0.02M sodium citrate buffer (pH 4.7). An initial and a final sample were applied to the paper (Whatman no. 3 MM) in two separate spots at the cathode end of the strip in the same way as in the paper-chromatography experiments; a current of 9–10 ma (about 10v/cm.) was applied for 6 hr. The spots were located by the photoprint method, cut out, eluted and estimated spectrophotometrically, as before. A result is shown in Table 13.

unlikely that the effect can be attributed to the chelating properties of ATP, since it cannot be reproduced by other chelating agents.

The affinity of ATP for the enzyme is lower than that of most true coenzymes, a concentration of 3–4 mM being required for full saturation. The activity of the deamination reaction is therefore variable within a concentration range of ATP corresponding to its physiological concentration range in animal tissues. If the concentration of ATP is low, the deamination of AMP will be slow and more AMP will remain available for rephosphorylation to ATP. A high ATP concentration will have the reverse effect. This is an example of a biochemical feed-back mechanism.

According to Lyubimova & Matlina (1954) the adenylic deaminase associated with myosin is also activated by ATP. It is possible therefore that the

phenomenon is not restricted to the adenylic deaminase of brain.

The observation that the enzyme is associated with insoluble particles is interesting in view of the adenylic deaminase activity of brain mitochondria (Weil-Malherbe & Green, 1955). Abood & Gerard (1954) found that in peripheral nerve of the rat adenylic deaminase is mainly associated with small microsomes of less than 100 m μ . in diameter.

SUMMARY

1. Adenylic deaminase of ox brain was purified 10- to 20-fold by fractionating the aqueous extract of acetone powder with ammonium sulphate and alumina gel C γ .

2. The activity is associated with insoluble particles which can be separated on the high-speed centrifuge. Treatment with sodium cholate, though inhibitory, produces some solubilization, as shown by the appearance of activity in the supernatant solution.

3. Under standard conditions the preparation is practically free of adenosinetriphosphatase, 5-nucleotidase, myokinase and adenosine deaminase activities.

4. The activity of the enzyme is greatly increased by adenosine triphosphate. The effect is specific; adenosine diphosphate, inosine tri- and diphosphates are inactive. Some activity persists even in complete absence of adenosine triphosphate. This activity cannot be accounted for by adenosine deaminase action.

5. The effect of varying adenosine triphosphate concentrations on the activity of the enzyme is independent of the concentration of adenylic acid.

6. The pH optimum is 6.2-6.8.

7. The enzyme is inhibited by some multivalent cations and by phosphate and fluoride. Other inhibitors were tested.

8. Adenosine triphosphate is recovered unchanged at the end of the reaction, while adenylic acid is converted into inosinic acid and ammonia.

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The Use of Paper Chromatography for *in vitro* Studies of Lactose Metabolism in Mammary-gland Preparations

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A major problem in the investigation of lactose synthesis by mammary-gland preparations is the estimation of lactose in the presence of other sugars, chiefly glucose. Previous workers (Grant, 1935, 1936; Knodt & Petersen, 1945; Malpress & Morrison, 1950; Hills & Stadie, 1952; Reithel, Horowitz, Davidson & Kittinger, 1952) have all used selective fermentation, combined with

chemical methods more or less specific for the carbohydrates concerned. The development of paper partition chromatography of sugars (Partidge, 1948) has given the opportunity of carrying out a preliminary physical separation of lactose.

In the work described here quantitative paper chromatography was so adapted that lactose could be estimated in the contents of Warburg vessels in