where pentose requirement may be decreased. Vardanis & Hochster (1961) found that the ratio of glucose 6-phosphate-dehydrogenase to 6-phosphogluconate-dehydrogenase activity in *Agrobacterium tumefaciens* was 8:1, a factor favouring the Entner–Doudoroff pathway over the pentose phosphate cycle. A similar situation may exist in older-growth-phase cells of *A. xylinum*.

SUMMARY

1. A mathematical model is presented for the metabolism of specifically ^{14}C-labelled glucose by the cellulose bacterium, *Acetobacter xylinum*.

2. The analysis treats the case of the pentose phosphate cycle in conjunction with the Entner–Doudoroff system and glucose resynthesis from triose phosphate generated by both pathways. In addition, triose phosphate and fructose 1,6-diphosphate but not fructose 6-phosphate can be converted glycolytically into pyruvate.

3. Recycling of glucose 6-phosphate via the pentose phosphate cycle and drainage of hexose phosphate into cellulose and oxogluconates is taken into account.

4. Sets of theoretical curves are obtained which show the molar specific activity of pyruvate molecules (relative to substrate glucose) arising from [^{14}C]glucose labelled at C-1, C-2 or C-6 as a function of various percentage participations of the pentose phosphate-cycle pathway and triose recombination. Theoretical curves are given for the percentage distribution of label in pyruvate derived from specifically ^{14}C-labelled glucose as a function of different pathway percentages.

5. The pentose phosphate cycle appears to be more active in young cells. About 80–90% of the triose phosphate formed via the pentose phosphate-cycle and Entner–Doudoroff pathways appears to recombine via fructose 1,6-diphosphate to yield hexose monophosphate.

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Adenosine Triphosphate–Nicotinamide Mononucleotide Adenylyltransferase of Pig-Liver Nuclei

EXTRACTION AND PURIFICATION OF THE ENZYME

BY M. R. ATKINSON, J. F. JACKSON* AND R. K. MORTON†
Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide, South Australia

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In previous work (Atkinson, Jackson & Morton, 1961) a partially purified preparation of ATP–NMN adenylyltransferase (EC 2.7.7.1) was used to determine the influence of pH and NMN concentration on the rate of formation of NAD. For further work on the specificity of this enzyme, a preparation of higher specific activity, and free of possible interfering enzymes, was needed.

The present paper describes the large-scale preparation of pig-liver nuclei, the preliminary removal of inactive protein, and the extraction of enzyme

* Present address: Department of Biochemistry, Stanford University, U.S.A.

† Deceased 27 September, 1963.

and its subsequent purification. A reproducible method was developed for obtaining the adenylyltransferase free of nucleic acid and with a specific activity of 2–3 μmoles of NAD formed/min./mg. of protein. A coupled assay for detection of this enzyme after electrophoresis in starch gel showed the presence of more than one active component. Some inactive protein was present in the best preparations.

EXPERIMENTAL

Procedure for large-scale purification of enzyme

Extraction and preliminary purification. Pig liver was cut into 1 in. cubes and packed in ice at the slaughter-house. Except where otherwise indicated, all subsequent procedures were carried out at 2°. Within 1 hr., portions (100 g.) of liver were cut into small portions with scissors, suspended in portions (400 ml.) of 0·25M-sucrose–3 mM-$CaCl_2$ and blended in a Waring Blendor (110v model). The operating voltage of the blender was kept at 90v for 30 sec. and at 70v for 3–5 min. with a constant-voltage transformer. The homogenate was strained through cheese-cloth. Portions (4 l.) of the homogenate were centrifuged at 1000*g* for 20 min. The loosely packed precipitate was resuspended in the sucrose–$CaCl_2$ solution (final vol. 4 l.) and centrifuged as before. The precipitate, which largely consisted of nuclei, was suspended in 0·25M-sucrose–15 mM-NaCl–10 mM-Na_2HPO_4 (final vol. 3·6 l.) and stirred for 30 min. at 20–25°. The precipitate obtained by centrifuging at 1000*g* for 20 min. was suspended in a solution (final vol. 1·2 l.) of Na_2HPO_4 (final concn. 0·2M). After 3–5 min. of rapid stirring at 20–25°, the solution became viscous owing to extraction of nucleoprotein. After being stirred for a further 60 min. at 20–25°, the dispersion was centrifuged at 3500*g* for 60 min. at 2°. The supernatant (about 1·2 l./kg. of liver) (phosphate extract; cf. Table 1) was kept at −15°; batches of 10–40 l. were used in subsequent purification. The nuclei from 1·6 kg. of pig liver were conveniently prepared and extracted in a day.

The supernatant (pH 8·5) obtained after centrifuging the thawed extract at 1000*g* for 20 min. was treated with 0·2 vol. of 0·2M-$CaCl_2$ with constant stirring during 30 min.; the pH fell to 7·4. The calcium phosphate gel was collected by centrifuging at 2000*g* for 20 min. and was suspended in a volume of 0·5M-NaCl–20 mM-sodium phosphate (pH 6·4) equal to the volume of $CaCl_2$ solution used, and was stirred for 30 min. and centrifuged as described above. The washed gel was stirred for 1 hr. in 0·21M-$(NH_4)_2SO_4$–80 mM-Na_2HPO_4–0·5 mM-EDTA (0·55 vol./vol. of $CaCl_2$ solution) and centrifuged (eluate 1; cf. Table 1). Four further eluates were obtained by similar extractions, each for 6–12 hr.

The first eluate was discarded, and the subsequent eluates (Table 1) were combined and brought to 28% saturation with $(NH_4)_2SO_4$. The supernatant obtained after centrifuging either at 4000*g* for 45 min. or, in later work, at about 30000*g* for 20 min., was brought to 45% saturation with $(NH_4)_2SO_4$. The precipitate was collected and dissolved in a minimum volume of water [$(NH_4)_2SO_4$ fraction; Table 1]. This fraction contained 41% of the activity of the phosphate extract with a 78-fold increased specific activity. Similar results were obtained with other batches of phosphate extract from the nuclei of 13–33 kg. of liver.

Further purification procedures

Chromatography on diethylaminoethylcellulose. The $(NH_4)_2SO_4$ fraction (Table 1) contains a considerable proportion of nucleic acid which may be removed by chromatography on diethylaminoethylcellulose (DEAE-cellulose) at pH 6·5. Approx. 40 ml. of the $(NH_4)_2SO_4$ fraction was mixed with 5 ml. of 0·2M-potassium phosphate, pH 6·5, and passed through a column (12 cm. × 5 cm.^{2}) of DEAE-cellulose in 25 mM-potassium phosphate, pH 6·5. The enzyme was eluted with 25 mM-potassium phosphate, pH 6·5, and the best fractions, collected between 45 and 80 ml., had $E_{280\,m\mu}/E_{260\,m\mu}$ ratios of approx. 1·5 and specific activities of 0·8–1·0; 68% of the activity was recovered.

A portion of the $(NH_4)_2SO_4$ fraction (9 ml.; Table 1) was dialysed against 10 mM-sodium phosphate, pH 7·5, centrifuged to remove an insoluble precipitate and applied to a column (11 cm. × 2·5 cm.^{2}) of DEAE-cellulose in 10 mM-sodium phosphate, pH 7·5. The column was washed with 3 ml. of 10 mM-sodium phosphate, pH 7·5, and with 60 ml. of 80 mM-NaCl–10 mM-sodium phosphate, pH 7·5, without eluting the adenylyltransferase. The enzyme was eluted with a linear gradient from 80 mM- to 200 mM-NaCl in 10 mM-sodium phosphate, pH 7·5, and the adenylyltransferase activity was obtained in two peaks at 100 mM- and 120 mM-NaCl. The most active fraction (at about 120 mM-NaCl) had a specific activity of 2·3, but the total recovery of enzyme was only about 50%.

Table 1. *Partial purification of the adenylyltransferase from a phosphate extract of nuclei from 14 kg. of pig liver*

Experimental details are given in the text. For conditions of assay see Atkinson *et al.* (1961).

Treatment	Vol. (ml.)	Enzyme activity (units)	$E_{280\,m\mu}/E_{260\,m\mu}$ ratio	Protein (mg.)	Specific activity (unit/mg.)
Phosphate extract	17500	730	0·74	108400	0·0067
Eluate 1	1360	22	1·15	1960	0·011
Eluate 2	1880	175	1·08	2400	0·073
Eluate 3	1260	114	1·02	1220	0·093
Eluate 4	1380	89·5	0·97	883	0·10
Eluate 5	1420	53	0·91	795	0·067
Eluates 2–5 combined	5940	432	1·0	5298	0·082
$(NH_4)_2SO_4$ fraction	37	300	1·3	574	0·52

Table 2. *Acetone fractionation of the adenylyltransferase*

Experimental details are given in the text. The starting material contained 125 units of the adenylyltransferase and 121 mg. of protein in 105 ml. of 25 mM-potassium phosphate–0·01 mM-EDTA, pH 6·5.

	Material soluble in 25 mM-phosphate, pH 6·5		Material soluble in 0·1 M-Na_2HPO_4	
Acetone (%, v/v)	Enzyme activity (units)	Specific activity (units/mg.)	Enzyme activity (units)	Specific activity (units/mg.)
0–15	3·35	2·2	9·0	2·7
15–20	3·3	1·2	2·6	1·9
30–50	15·4	—	10·8	—
>50	0·5	—	—	—

Acetone fractionation. The enzyme which had been passed through DEAE-cellulose at pH 6·5 was dialysed against 25 mM-potassium phosphate–0·01 mM-EDTA, pH 6·5, and fractionated with acetone at −2 to −10°. The fractions which were precipitated at 15, 30 and 50% (v/v) of acetone were suspended in 25 mM-potassium phosphate, pH 6·5, and dialysed against this buffer. After removal of the supernatant (Table 2), the residue in each fraction was dissolved in 0·1 M-Na_2HPO_4 (Table 2). The material that was precipitated at 15% (v/v) acetone which was insoluble in 25 mM-phosphate but soluble in 0·1 M-Na_2HPO_4 had a specific activity of 2·7. In one experiment, the addition of acetone to 15% (v/v) precipitated crystalline material as rectangular plates, together with amorphous material. The crystals were insoluble in 25 mM-potassium phosphate and soluble in 0·1 M-Na_2HPO_4; the specific activity was about 2·3. In subsequent experiments, only amorphous material was obtained in this fraction and it cannot be concluded that the crystals were those of the adenylyltransferase. The preparation at this stage contained components with $S_{20,w}$ values of approx. 4·2 s and 8·5 s in 0·1 M-NaCl–20 mM-tris–0·1 mM-EDTA, adjusted to pH 8·0 with HCl.

In one experiment, 105 units of adenylyltransferase (specific activity 0·47) were fractionated with acetone without prior treatment with DEAE-cellulose. Approx. 10 units of activity were recovered in the 0–16·5% (v/v) acetone fraction with a specific activity of 3·3.

Starch-gel electrophoresis. Samples of the enzyme were subjected to electrophoresis in starch gel by a modification of Poulik's (1957) method. The gel was prepared from 12 g. of hydrolysed starch (Connaught Medical Laboratories, Toronto, Canada) in 100 ml. of 0·09 M-tris–0·5 mM-EDTA adjusted to pH 8·25 with citric acid. The electrolyte vessels contained 0·3 M-boric acid adjusted to pH 8·25 with NaOH. The enzyme was placed in the gel on Whatman 3MM paper and electrophoresis was carried out for 9–10 hr. at 2° at about 3 v/cm.

Protein was detected with Amido Black in acetic acid–methanol (Smithies, 1955). Adenylyltransferase activity was detected as follows. A freshly prepared mixture of equal volumes of 8 mM-ATP–2 mM-NMN–32 mM-$MgCl_2$–0·4 M-glycylglycine, pH 7·6, and 0·5 M-ethanol–0·2 M-tris–HCl, pH 9·5, was mixed with 1·5 mg. of nitroblue tetrazolium/ml., 25 µl. of a 1% solution of pig-heart diaphorase (Massey, Gibson & Veeger, 1960) and 75 µl. of 0·3% yeast alcohol dehydrogenase immediately before use. A piece of Whatman no. 1 filter paper was soaked in this solution and applied to the cut surface of the gel. In 10–20 min. regions of ATP–NMN-adenylyltransferase activity were detected by the formation of intense purple bands of formazan, due to the coupled reactions:

$$\text{ATP} + \text{NMN} \xrightarrow{\text{Adenylyltransferase}} \text{NAD} + \text{pyrophosphate}$$

$$\text{NAD} + \text{C}_2\text{H}_5\cdot\text{OH} \xrightarrow{\text{Alcohol dehydrogenase}} \text{NADH}_2 + \text{CH}_3\cdot\text{CHO}$$

$$\text{NADH}_2 + \text{nitroblue tetrazolium} \xrightarrow{\text{Diaphorase}} \text{NAD} + \text{formazan}$$

The enzyme was then inactivated by dipping the paper in acetic acid–methanol–water (1:5:5, by vol.). Regions of the starch gel corresponding to adenylyltransferase activity were marked and the gel was then soaked in acetic acid–methanol–water, as was the slice which had been stained for protein. In this way, both slices were subject to similar shrinkage effects and the distribution of enzyme could be compared with that of protein.

A sample of adenylyltransferase freshly prepared as described in Table 1 was dialysed against 5 mM-sodium pyrophosphate adjusted with HCl to pH 8·0, and fractionated with acetone. Material that was precipitated between 40 and 60% (v/v) was dialysed against the same buffer and fractionated with $(NH_4)_2SO_4$. Material that was precipitated (specific activity 1·05) between 38 and 50% saturation was dialysed against the pyrophosphate buffer and centrifuged at 259000*g* for 45 min. (Spinco model E ultracentrifuge). The supernatant contained two main bands of enzymic activity (sample A in Fig. 1). A sample of enzyme prepared by acetone fractionation of an $(NH_4)_2SO_4$ fraction (cf. Table 1) that had been kept at −15° for 6 months was centrifuged at 259000*g* for 30 min. On electrophoresis, this sample (specific activity 0·62; sample B in Fig. 1) showed one main region of activity. A mixture of samples A and B showed at least three regions of adenylyltransferase activity with mobilities corresponding to those of samples A and B (Fig. 1). All the regions of adenylyltransferase activity correspond to regions that contain protein (Fig. 1), but after electrophoresis each preparation showed protein bands in regions devoid of adenylyltransferase activity. In other experiments, it was shown that centrifuging at 259000*g* removed protein that only partly entered the gel from the sample slot and that some adenylyltransferase activity was associated with this protein.

Other chromatographic studies

(*a*) *With carboxymethylcellulose.* The enzyme was retained by carboxymethylcellulose in 0·01 M-sodium acetate–0·01 mM-EDTA, pH 5·0, and only 25% of the activity was

recovered on elution with M-NaCl–0·01 mM-EDTA. There was no increase in specific activity. At pH 6·5 little of the activity was retained by the carboxymethylcellulose.

(*b*) *With Amberlite CG-50.* The adenylyltransferase in 20 mM-sodium phosphate, pH 5·8, was completely retained by a column of this resin. No activity was recovered by elution with 0·04M-sodium phosphate, pH 6·5, and 0·04M-sodium phosphate, pH 7·0. About 11% of the adenylyltransferase was recovered by elution with 0·2M-sodium phosphate, pH 7·0, but the specific activity was only increased from 0·26 to 0·32. When the adenylyltransferase (43 units; specific activity 0·28) in 50 mM-sodium phosphate, pH 7·0, was applied to a column (13·3 cm. × 1·5 cm.^{2}) of the resin in 50 mM-sodium phosphate, pH 7·0, about 22 units of the enzyme passed through the column (specific activity 0·18) and 13 units (specific activity 0·6) were eluted with 0·2M-sodium phosphate, pH 7·0.

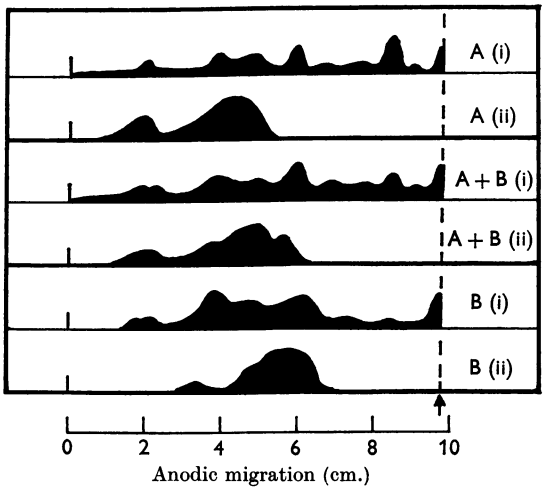

Fig. 1. Distribution of protein and adenylyltransferase activity after electrophoresis in starch gel. Details of the preparation of the adenylyltransferase samples A and B and of detection of protein (i) and adenylyltransferase activity (ii) in A, B and a mixture (A + B) are given in the text. The arrow marks the position of the borate–citrate boundary.

When 3 ml. of a solution containing 9·2 units of enzyme (specific activity 0·3) was dialysed against 6·1M-urea–0·15M-sodium phosphate, pH 6·15, and applied to a column (15·2 cm. × 1·5 cm.^{2}) of Amberlite CG-50 in the same buffer, inactive protein passed through the column and further elution with urea–phosphate removed 3·9 units of adenylyltransferase with specific activities of 0·5–0·6. The enzyme showed no loss of activity in this urea–phosphate solution during several days.

(*c*) *With calcium phosphate.* A solution containing 19·3 units of the adenylyltransferase ($E_{280\,m\mu}/E_{260\,m\mu}$ ratio 1·37; specific activity 0·98) in 5 ml. of 10 mM-potassium phosphate, pH 6·6, was passed through a column (2·8 cm. × 1·1 cm.^{2}) of calcium phosphate (Main, Wilkins & Cole, 1959), the column was eluted with 0·35M-potassium phosphate, pH 6·6, and 94% of the enzyme was recovered with a specific activity of 1·6 ($E_{280\,m\mu}/E_{260\,m\mu}$ ratio 1·46). After fractionation with $(NH_4)_2SO_4$ between 35% and 45% saturation, this fraction was chromatographed on a similar calcium phosphate column, which was eluted successively with 0·1M-, 0·2M- and 0·35M-potassium phosphate, pH 6·6. About 75% of the enzyme was recovered, mainly in the last fraction. The best fraction had a specific activity of 1·7 and an $E_{280\,m\mu}/E_{260\,m\mu}$ ratio of 1·59.

Stability in urea

There was no loss of activity when the enzyme was dialysed against 5M-urea–50 mM-sodium phosphate, pH 6·0, for 48 hr. at 2°. On subsequent dialysis against 50 mM-sodium phosphate, pH 7·0, inactive protein was precipitated and the specific activity of the soluble fraction increased from about 0·3 to 0·8.

On fractionation of a sample of transferase with $(NH_4)_2SO_4$ in 2·0M-urea–0·5M-K_2HPO_4 between 25% and 45% saturation, an increase in specific activity from 0·07 to 0·61 was obtained with a recovery of 69% of the activity. In the absence of urea, the recovery in this fraction was only 30%, and some adenylyltransferase activity was precipitated at 25% saturation.

Extraction

Experiments were carried out with acetone-dried liver powder, and with the nuclear fraction prepared essentially as described above. Table 3 summarizes results obtained with acetone-dried powders. The highest specific activities

Table 3. *Extraction of the adenylyltransferase from acetone-dried powder of pig liver*

Portions of acetone-dried powder (25 g.) from 75 g. of fresh liver (Kornberg, 1950) were stirred at 20–25° with 250 ml. of the solutions indicated. Adenylyltransferase activity and protein were measured in the supernatant obtained by centrifuging at 4000*g* for 20 min.

		Properties of supernatant			
Extracting solution	Time (min.)	$E_{280\,m\mu}/E_{260\,m\mu}$ ratio	Protein (g.)	Enzyme (units)	Specific activity (unit/mg.)
0·1M-Na_2HPO_4	10	0·85	6·4	9·35	0·0015
	30	0·89	10·5	10·5	0·0010
0·05M-KCl	10	0·92	5·5	0	—
	30	0·90	7·65	1·35	0·0002
Water	10	0·90	5·64	0	—
0·01M-Na_2CO_3	10	0·86	6·4	0	—
0·1M-Na_2HPO_4 after 0·05M-KCl	10	0·69	1·7	3·83	0·0023
0·1M-Na_2HPO_4 after water	10	0·68	2·1	2·86	0·0014

Table 4. *Extraction of the adenylyltransferase from the nuclear fraction of pig liver*

Nuclei were prepared essentially as described in the text except that the medium for blending and washing of the nuclei contained 0·01 M-triethanolamine–HCl, pH 7·2, and the extraction of the pellet with the sucrose–NaCl–Na_2HPO_4 solution was omitted. Portions (5 ml.) of the final suspension, which had been kept at −15° for 3 days, were mixed with an equal volume of the required solution to give the final concentrations shown. Adenylyltransferase activity and protein were measured in the supernatant obtained after stirring for 30 min. at 20–25° and centrifuging at 4000*g* for 20 min. at 2°.

Extracting solution	Properties of supernatant: $E_{280\,m\mu}/E_{260\,m\mu}$ ratio	Protein (mg.)	Enzyme (unit)	Specific activity (unit/mg.)
0·25 M-Na_2HPO_4	0·64	70	0·23	0·0033
3 M-Urea–12·5% satd. $(NH_4)_2SO_4$, pH 7·0	0·61	70	0·22	0·0031
3 M-Urea, pH 7·0	0·64	57	0·20	0·0035
12·5% satd. $(NH_4)_2SO_4$, pH 7·0	0·62	62	0·19	0·0031
25% satd. $(NH_4)_2SO_4$, pH 7·0	0·65	66	0·11	0·0017
25% satd. $(NH_4)_2SO_4$–0·25 M-Na_2HPO_4	0·63	54	0·05	0·0009
Sucrose–$CaCl_2$–triethanolamine (blending medium)	0·79	27	0·05	0·0019
Water	0·80	36	0·03	0·0008

Table 5. *Extraction of the adenylyltransferase from the nuclear fraction of pig liver with detergent*

Nuclei were prepared and extracted as described in Table 4. Detergent concentrations are given as % (v/v) of concentrated sodium dodecyl sulphate (Supreem 410; J. R. Alexander and Son Ltd., Adelaide).

Concn. of detergent (%, v/v)	$E_{280\,m\mu}/E_{260\,m\mu}$ ratio	Protein (mg.)	Enzyme (unit)	Specific activity (unit/mg.)
0	0·75	27	0·065	0·0024
0·10	0·73	38	0·077	0·0020
0·25	0·73	45	0·117	0·0026
0·50	0·68	54	0·143	0·0026
1·0	0·72	72	0·192	0·0027
2·5	0·76	123	0·163	0·0013

obtained were 0·0015 (125 units/kg. of fresh liver) with 0·1 M-Na_2HPO_4, and 0·0023 (51 units/kg. of fresh liver) with 0·1 M-Na_2HPO_4 after extraction with 0·05 M-KCl.

In numerous preliminary experiments, the most satisfactory extraction of enzyme from the nuclear fraction was obtained with M-NaCl (specific activity 0·0058), and with 0·25 M-Na_2HPO_4 (specific activity 0·017).

A nuclear fraction that had been frozen for 3 days was extracted with a number of reagents. Table 4 summarizes results obtained. The highest yield of enzyme was obtained with 0·25 M-Na_2HPO_4, but the specific activity (0·0033) was lower than that obtained with freshly prepared nuclei. The only reagent that gave a higher specific activity in the first extract was 3 M-urea.

It was also found that the enzyme could be extracted from nuclei with an alkyl sulphate detergent. Table 5 shows the effect of concentration of detergent on yields and specific activities. As observed with other treatments, the extracts of enzyme always contained nucleic acid.

DISCUSSION

Commencing with an acetone-dried powder of pig liver, Kornberg (1950) prepared an extract that contained ATP–NMN adenylyltransferase with a specific activity of 0·002 unit/mg. of protein. By fractionation with ammonium sulphate and adsorption on calcium phosphate, adenylyltransferase of specific activity 0·21 was obtained. In our preliminary studies, attempts were made to effect a further purification by fractionation with ammonium sulphate, acetone or ethanol at several pH values. It was observed that the enzyme was not precipitated by as narrow a range of concentration of precipitants as is usual with soluble proteins. As little useful enrichment could be obtained in this way, advantage was taken of previous observations that the adenylyltransferase is associated with the nuclear fraction of liver cells (Hogeboom & Schneider, 1952; Branster & Morton, 1956).

When suitable speeds and times of blending had been established by experiment, the nuclei obtained in these large-scale preparations were little inferior to those prepared on a small scale in a Potter–Elvehjem-type homogenizer (cf. Branster & Morton, 1956). Extraction of these nuclei with solutions of low ionic strength removed much interfering protein with little loss of enzyme (cf. the Experimental section). The removal of contaminating erythrocytes and haemoglobin by stirring in 0·25 M-sucrose–15 mM-sodium chloride–10 mM-disodium hydrogen phosphate was a considerable advantage.

It was not found possible to extract the adenylyltransferase from washed nuclei in useful yield without simultaneous extraction of much nucleic acid, as evidenced by the low $E_{280\,m\mu}/E_{260\,m\mu}$ ratios of the extract (Tables 4 and 5). Irrespective of the extent of mechanical damage to the nuclei, the adenylyltransferase was not extracted in solutions of low ionic strength. Of the solutions that were found to extract the adenylyltransferase, 0·2 M-disodium hydrogen phosphate was chosen for routine use since it served to generate calcium phosphate gel on the addition of calcium chloride. The adenylyltransferase was adsorbed on this gel and thus was readily collected from the large volumes of phosphate extract. Most of the nucleic acid was removed during the washing and subsequent elution of the enzyme. Unless this preliminary removal of nucleic acid was effected, subsequent treatment with DEAE-cellulose was not satisfactory. From small batches of nuclei, the enzyme was sometimes obtained in association with deoxyribonucleic acid by dilution of extracts in M-sodium chloride (Hogeboom & Schneider, 1952). However, the results were not reproducible and the large volumes involved made this method unsuitable for large-scale work. On treatment of nuclear extracts with protamine the adenylyltransferase was bound in the precipitate and could not be recovered without extraction of nucleic acid.

The procedure described (Table 1) was reproducible, as was removal of residual nucleic acid with DEAE-cellulose at pH 6·5. Acetone fractionation of the adenylyltransferase at this stage gave a product with specific activities of 2–3 on several occasions. Extracts of acetone-dried powder of the whole liver had less than a thousandth of the purity of the adenylyltransferase at this stage.

After electrophoresis in starch gel, more than one zone of adenylyltransferase activity was found and the best preparations contained inactive protein; some of this protein may have arisen by denaturation in the starch-gel procedure. The electrophoretic differences between the components of a fresh and a stored preparation of adenylyltransferase (Fig. 1) are at present unexplained.

The stability of the adenylyltransferase in 5 M-urea is potentially useful for the removal of less-stable proteins, but there is ample evidence that proteins interact with cyanate which is formed in urea solutions (Stark, Stein & Moore, 1960), and enzyme so modified may have different kinetic properties from those of the native material. None of the enzyme used in the kinetic studies for which the preparation was required was treated with urea.

Although the final product obtained as described above may not be homogeneous, there was no evidence of contaminants that interfered with kinetic or thermodynamic studies of the adenylyltransferase reaction.

SUMMARY

1. Conditions for the extraction of adenosine triphosphate–nicotinamide mononucleotide adenylyltransferase from pig-liver nuclei have been described. Extracts that contained the adenylyltransferase also contained nucleic acid.

2. Adenylyltransferase that would form 2–3 μ-moles of nicotinamide–adenine dinucleotide/min./mg. of protein was obtained by adsorption on calcium phosphate, fractionation with ammonium sulphate, chromatography on ion-exchange cellulose and fractionation with acetone. The final product had an $E_{280\,m\mu}/E_{260\,m\mu}$ ratio of 1·5–1·6.

3. After electrophoresis in starch gel at least three zones with adenylyltransferase activity were detected in a coupled assay with nicotinamide mononucleotide, adenosine triphosphate, magnesium chloride, ethanol, alcohol dehydrogenase, diaphorase and nitroblue tetrazolium. Inactive protein was found in the purest preparations of the adenylyltransferase.

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