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ADENOSINE TRIPHOSPHATE: NICOTINAMIDE MONONUCLEOTIDE ADENYLYLTRANSFERASE OF PIG LIVER

PURIFICATION AND PROPERTIES*

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Summary

Adenosine triphosphate : nicotinamide mononucleotide adenylyltransferase (EC 2.7.7.1) has been purified approximately 3500-fold from an extract of pig liver nuclei to a specific activity of 40 μmol of NAD^+ per min per mg protein. The enzyme was found to have a molecular weight of 203 000, a frictional ratio of 1.6 and an isoelectric point of approximately 5. Michaelis constants for ATP and NMN were 0.11 mM and 0.12 mM, respectively.

Introduction

ATP : NMN adenylyltransferase (EC 2.7.7.1) catalyzes the transfer of the adenylyl moiety of ATP to the phosphoryl group of NMN to form NAD^+:

$$ATP + NMN \rightleftharpoons NAD^+ + PP_i$$

This is the final reaction in what is now believed to be the major pathway for the biosynthesis of NAD^+ in mammals [2—4]. The reaction is freely reversible and requires Mg^{+2}.

Mammalian NMN adenylyltransferase has been partially purified from pig and rat liver. Kornberg [5], starting from an acetone powder of pig liver, purified the enzyme 100-fold to a specific activity of approximately 0.2 μmol of NAD^+ synthesized per min per mg of protein. Atkinson et al. [6] purified the enzyme from pig liver using the isolation of nuclei as a first step. The

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specific activity of their purest preparation was approximately 3, but the yield of enzyme was very low. Traub et al. [7] purified the enzyme from rat liver nuclei to a specific activity of 1.3. NMN adenylyltransferase has also been partially purified from non-mammalian sources including chicken erythrocytes [8], yeast [5,9], *Escherichia coli* [9], and *Lactobacillus fructosus* [10].

Two observations have made further investigation of this enzyme of particular interest:

1. In most eukaryotic cells the enzyme is found exclusively in the cell nucleus [11—16, Kuehl, L. (1974) unpublished observations].
2. A number of investigators have reported a correlation between ATP: NMN-adenylyltransferase activity and DNA synthesis [12,17—21].

In this report we describe a method for purification of the transferase from pig liver nuclei. A 3500-fold purification of the enzyme has been achieved, yielding preparations with specific activities as high as 40. Although homogeneity has not been achieved, this is the most extensively the transferase has been purified from any source. We also report on various physical and kinetic properties of the pig liver enzyme.

Materials and Methods

Reagents

Yeast alcohol dehydrogenase, bovine pancreatic chymotrypsinogen A and beef liver catalase for sedimentation studies were obtained from Worthington Biochemical Corp. Rabbit muscle lactic dehydrogenase, bovine thyroglobulin, horse cytochrome *c*, and beef liver catalase for Stokes radius determination were obtained from Sigma Chemical Co. Bovine serum albumin was from Schwarz/Mann, protamine sulfate (salmine, A grade) from Calbiochem, and chicken egg albumin from Pentex.

Enzyme assays

NMN adenylyltransferase was assayed essentially as described by Kornberg [5] except that the final concentration of ATP in the reaction mixture was increased to 4 mM and the incubation time was decreased to 5 min (3 min for the kinetic studies). One unit of enzyme was taken to be that amount which catalyzes the synthesis of 1 μmol of NAD^+ per min under the assay conditions employed. The assay was linear up to a ΔA_{340} value of 0.3 (0.123 units of enzyme per ml).

Catalase was assayed spectrophotometrically by the method of Beers and Sizer [22], and alcohol dehydrogenase was determined essentially as described by Vallee and Hoch [23].

Protein determination

Protein concentrations were determined by the method of Lowry et al. [24], using bovine serum albumin as a standard.

DNA determination

The sample to be analyzed was made 7.14% in trichloroacetic acid and the suspension centrifuged. The pellet was suspended in 5% trichloroacetic acid

heated at 90°C for 15 min. The sample was then centrifuged, and the DNA concentration in the supernatant determined by the method of Burton [25].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Reduction and alkylation of proteins and subsequent electrophoresis on sodium dodecyl sulfate-polyacrylamide gels were done as described by Weber et al. [26]. Proteins were denatured with guanidine-HCl. the gels were 10% in acrylamide. A log molecular weight versus mobility plot for several standard proteins was linear and was used to estimate the molecular weights of unknown polypeptides [26].

Stokes radius determination

A 2.7 cm × 78.5 cm column of Sepharose 6B (Pharmacia), equilibrated with 50 mM Tris · HCl, pH 7.5/0.1 M NaCl/0.5 mM dithiothreitol at 4°C was calibrated with a series of proteins of known Stokes radii [27]. The Einstein-Stokes equation was used to calculate Stokes radii from the diffusion coefficients ($D_{20,w}$) with the following results: bovine pancreas chymotrypsinogen A, 22.6 Å [28]; bovine serum albumin, 35.7 Å [29,30]; rabbit muscle lactic dehydrogenase, 41.2 Å [31]; bovine liver catalase, 52.5 Å [32]; and bovine thyroglobulin, 85.6 Å [33]. NMN adenylyltransferase from a Sephadex G-200 column (see purification procedure) concentrated to approximately 15 mg protein and 4 units of transferase activity per ml was chromatographed in the following buffers, all of which contained 50 mM Tris · HCl and 0.5 mM dithiothreitol: 0.10 M NaCl, pH 7.5; 1.0 M NaCl, pH 7.5; 0.10 M NaCl/5 mM ATP/20 mM $MgCl_2$, pH 7.5; and 0.10 M NaCl, pH 8.5.

Density gradient sedimentation

Sucrose density gradients, isokinetic for particles with a partial specific volume of 0.725 cm^3 per g, were prepared for the Beckman type SW 41 rotor as described by Noll [34,35] and McCarty et al. [36]. The gradients, which were exponentially convex and 5% to 20.6% in sucrose, were made up in 10 mM Tris · HCl, pH 7.5. The enzyme sample was a hydroxyapatite fraction (see purification procedure) containing approximately 1.8 mg protein and 1.9 units of activity per ml. Beef liver catalase (0.4 mg/ml) and yeast alcohol dehydrogenase (0.3 mg/ml) were used as internal standards. Enzymes were dissolved in 10 mM Tris · HCl, pH 7.5/0.10 M NaCl/0.5 mM dithiothreitol; 0.2-ml samples were applied to the gradients. Centrifugation was at 40 000 rev./min for 10 or 24 h at 5°C.

Column electrophoresis

Electrophoretic mobility studies at various pH values were made with a vertical column electrophoresis apparatus [37]. The following buffers, all of which had an ionic strength of 100 mM and contained 0.5 mM dithiothreitol, were employed: 50 mM sodium succinate, pH 4.0/80.2 mM NaCl; 50 mM sodium acetate, pH 4.5/81.6 mM NaCl; 50 mM sodium acetate, pH 5.0/67.5 mM NaCl; 50 mM 2(*N*-morpholino) ethane sulfonic acid (MES)/NaOH, pH 6.0/79.4 mM NaCl; 50 mM imidazole/HCl, pH 7.0/76.8 mM NaCl; 50 mM Tris · HCl, pH 8.0/72.5 mM NaCl; 50 mM *N*,*N*-bis(2-hydroxyethyl)glycine

(bicine)/NaOH, pH 8.0/84.6 mM NaCl. Protein samples in 1 ml of electrophoresis buffer containing ^3H_2O, were applied to a 1 cm × 40 cm column of Bio-Gel A-150 M (1% beaded agarose; Bio-Rad) equilibrated with the same buffer. The column was then washed with buffer until the sample had moved to the middle of the gel. Electrophoresis was at 470 V for 17 h at 4°C, after which the column was eluted with buffer; the column effluent was collected in 1-ml fractions. The distance between the ^3H_2O peak and the protein peak was assumed to correspond to the distance which the protein had migrated in the electric field. The ^3H_2O served to mark the origin and to control for electroendosmosis. When ^3H_2O-containing protein samples were applied to the column and eluted without electrophoresis, the protein emerged slightly ahead of the ^3H_2O peak, indicating that the proteins were being partially excluded from the agarose gel. Corrections were made for this effect.

Results

Enzyme concentration in various tissues

Nuclei were isolated by methods similar to those described below. In a survey of rat tissues, liver nuclei had 2.5 and 7 times more transferase activity per g wet weight of tissue than did kidney or brain nuclei, respectively. In a comparison of the transferase activities in the NaCl extracts derived from rat, pig, and beef liver nuclei (see NaCl extraction procedure, below), pig liver yielded several times as many units of enzyme activity per g of tissue as rat or beef liver. On the basis of these results, pig liver was selected as the source of enzyme for further studies.

Purification

The purification procedure is summarized in Table I. The data given in the table and in the discussion which follows are averages of several enzyme preparations. All steps were carried out at 4°C unless otherwise stated. Dithiothreitol was found to stabilize enzyme activity, particularly during dialysis; all solu-

TABLE I

PURIFICATION OF PIG LIVER ATP-NMN-ADENYLYLTRANSFERASE

Fraction	Total protein (g)	Total units	Yield (%)	Specific activity (units/mg)
Homogenate	1680	1700*	100	0.0010
Nuclei	250	1140	67	0.0045
Heat	107	700	41	0.0065
First ethanol	19.5	453	26.6	0.023
Second ethanol	7.2	376	22.0	0.053
Sephadex G-200	0.68	227	13.3	0.334
Hydroxyapatite	0.173	179	10.5	1.03
pH	0.064	142	8.35	2.22
CM-cellulose	0.0034	77.5	4.55	23

* To correct for the NAD^+ present in the homogenates; the ΔA_{340} values obtained in enzyme assays from which NMN was omitted were subtracted from ΔA_{340} values obtained in standard enzyme assays.

tions in the latter part of the purification procedure contained 0.5 mM dithiothreitol. Preparations of NMN adenylyltransferase at various stages of purity could be stored at −27° C for several months with little loss of activity.

Sixteen kg of pig liver was homogenized in an equal volume of 0.25 M sucrose/50 mM Tris · HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$ for 15 s in a 1-gallon Waring blendor run at low speed and connected to a rheostat delivering 85 V. The homogenate was filtered through a series of perforated stainless steel plates, then through one layer of fine cheesecloth. The resulting filtrate was made up to 32 l with homogenization buffer and centrifuged 30 min at 1400 × *g* in a swinging-bucket centrifuge. The supernatant was discarded, and the pellets were suspended in 0.25 M sucrose/0.5% Triton X-100/1 mM $MgCl_2$ and made up to 32 l. This suspension was centrifuged as before and the supernatant discarded. The nuclear pellets were suspended in 0.25 M sucrose/50 mM Tris · HCl, pH 7.5/25 mM KCl/5 mM $MgCl_2$ and made up to 16 l.

The nuclear fraction was made 0.4 M in NaCl by the addition of 10.66 l of 1.0 M NaCl. This ionic strength gave optimal solubilization of the transferase. DNA was precipitated from this suspension by the addition of 5.33 liters of 1% protamine sulfate. Over 90% of the DNA present in the suspension was precipitated by this treatment.

The suspension from the preceeding step (volume, 32 l) was pumped through a stainless steel coil in which it was rapidly brought to 50° C, held at this temperature for 5 to 10 min, then quickly cooled to 2° C. The suspension was centrifuged at 1400 × *g* for 40 min. The pellet was discarded; the supernatant (volume, 30 l) was designated the heat fraction. Enzyme preparations could be heated at 50° C for up to 40 min and maintain full activity; at 60° C, however, activity was rapidly lost.

Five-liter portions of the heat fraction were cooled to 0° C, then brought to an ethanol concentration of 19% by the addition of 95% ethanol cooled to −70° C. The ethanol was added slowly with constant stirring. The temperature of the protein solution dropped to −8° C during the ethanol addition. The suspension was centrifuged at −8° C for 25 min at 19 600 × *g*. The supernatant was discarded and the pellets suspended in 0.1 M Tris · HCl, pH 7.5/0.35 M NaCl/0.5 mM dithiothreitol to a volume of 2 l. The suspension was stirred overnight at 0° C, then centrifuged at 19 600 × *g* for 25 min. The pellets were discarded.

The supernatant from the first ethanol fractionation (volume, approx. 1.9 l) was brought to an ethanol concentration of 19% as before, the resulting suspension was centrifuged at 19 600 × *g* for 25 min at −8° C and the supernatant discarded. The pellets were suspended in 20 mM Tris · HCl, pH 7.5/0.35 M NaCl/0.5 mM dithiothreitol, and made up to 360 ml. The suspension was centrifuged at 78 000 × *g* for 1 h at 0° C and the pellets were discarded.

The slightly turbid supernatant from the second ethanol fractionation (volume, approx. 290 ml) was applied to a 10.6 cm × 110 cm column of Sephadex G-200 equilibrated with 20 mM Tris · HCl, pH 7.5/0.35 M NaCl/0.5 mM dithiothreitol, and eluted with the same buffer. The elution volume for the enzyme was approx. 4.5 l. Fig. 1 shows a typical elution pattern. The large A_{280} peak had an elution volume equal to the void volume of the column

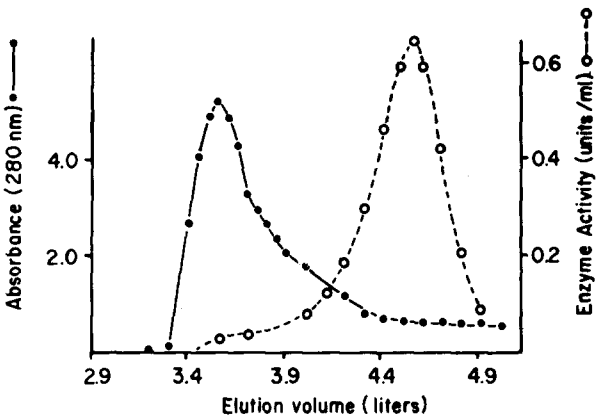

Fig. 1. Chromatography on Sephadex G-200. A second ethanol fraction (293 ml) containing 3.6 g of protein and 522 units of NMN adenylyltransferase activity was chromatographed on Sephadex G-200 as described in the text.

(approx. 3.5 l). The enzyme-containing fractions were pooled (vol. approx. 920 ml) and concentrated to 75 ml on a Diaflow ultrafiltration apparatus (Amicon) with an XM 50 membrane. When ethanol fractions that had been stored at −27° C were chromatographed on Sephadex G-200, a portion of the enzyme activity emerged in the void volume fractions. The extent to which enzyme activity became associated with this large molecular weight material was proportional to the storage time at −27° C. Thus, the best results were obtained when ethanol fractions were chromatographed immediately after preparation. The enzyme displayed a marked tendency to aggregate under a variety of conditions and the aggregation appeared to be concentration dependent.

The Sephadex G-200 fraction was dialyzed overnight against 0.1 M potassium phosphate, pH 6.9/0.5 mM dithiothreitol. The dialyzed solution was applied to a 1.5 cm × 21 cm hydroxyapatite column (Bio-Gel HT; Bio-Rad)

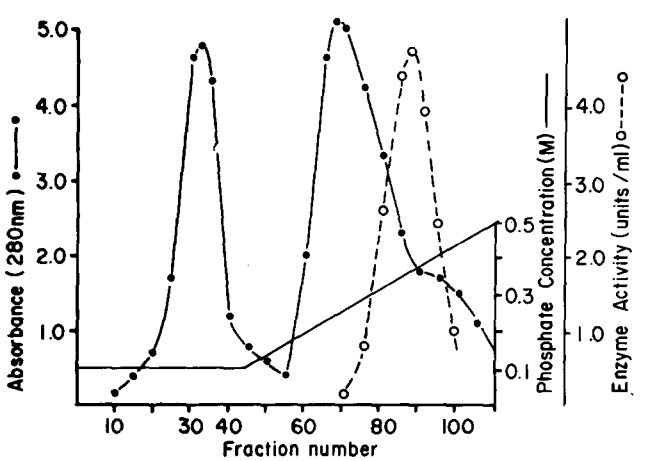

Fig. 2. Chromatography on hydroxyapatite. A Sephadex G-200 fraction (73 ml after dialysis) containing 0.639 g of protein and 225 units of NMN adenylyltransferase activity was chromatographed on a hydroxyapatite column as described in the text.

equilibrated with the same buffer. The column was washed with 60 ml of the equilibrating buffer. A linear potassium phosphate gradient containing 0.5 mM dithiothreitol was then started (0.10 M—0.50 M in phosphate, total volume 200 ml). Fig. 2 shows a typical chromatographic pattern. The enzyme was eluted at a potassium phosphate concentration of 0.28—0.40 M. Enzyme-containing fractions were pooled (approx. 95 ml).

The hydroxyapatite fraction was dialyzed for 15 h against several changes of 50 mM acetate, pH 5.0/0.22 M NaCl/5 mM $MgCl_2$ /0.5 mM dithiothreitol. A large precipitate formed. The suspension was centrifuged at 27 000 × *g* for 5 min; the supernatant was designated the pH fraction. The enzyme was stable over a wide pH range. No loss of activity was observed when a preparation, exposed for 10 h at 4°C to pH values ranging from 3.5 to 9.5, was subsequently assayed at neutral pH.

Portions of the pH fraction containing from 10—30 mg of protein were applied to a 0.9 cm × 21 cm CM-cellulose (Bio-Rad; 0.55 meq. per g) column equilibrated with 50 mM acetate, pH 5.0/0.22 M NaCl/5 mM $MgCl_2$ /0.5 mM dithiothreitol. The column was then washed with the following sequence of buffers, all at pH 5.0: 50 ml of the equilibrating buffer, 45 ml of equilibrating buffer containing 5 mM ATP, 35 ml of equilibrating buffer, and 250 ml of equilibrating buffer containing 0.3 M NaCl (0.52 M NaCl, designated the high-salt wash). Fractions containing the enzyme activity eluted by ATP (Fig. 3) were pooled (approx. 16 ml) and dialyzed for 4 h against saturated ammonium sulfate that contained 1 mM dithiothreitol. The suspension was centrifuged at 39 000 × *g* for 10 min, and the pellets dissolved in 0.5 ml of 10 mM Tris · HCl, pH 7.5/75 mM NaCl/0.5 mM dithiothreitol. This solution was applied to a 0.9 cm × 26 cm Sephadex G-50 column equilibrated with the same buffer to remove ATP from the enzyme. Eluate fractions containing protein were com-

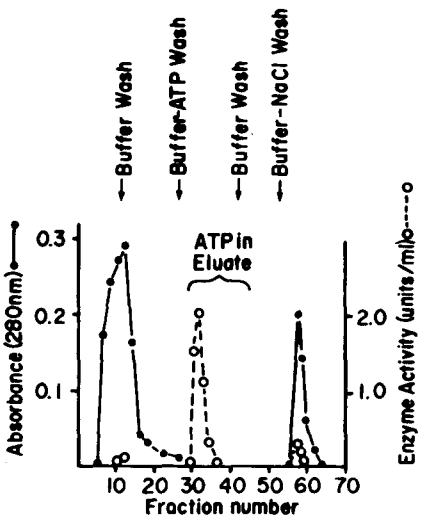

Fig. 3. Substrate elution from CM-cellulose. A pH fraction (26 ml) containing 12.5 mg of protein and 21.4 units of NMN adenylyltransferase activity was chromatographed on a CM-cellulose column as described in the text. The A_{280} values for fractions containing ATP were not determined.

bined (approx. 4 ml); this was designated the CM-cellulose fraction. The results from chromatography on CM-cellulose were quite variable; the proportion of the bound enzyme which could be eluted with ATP differed from one experiment to another, and we were unable to ascertain the reason for this variability. In a series of four experiments, the specific activities of the ATP-eluted material ranged from 12.5 to 40 with an average of 23 (Table I).

Comparison of the results obtained when the enzyme was eluted from CM cellulose with ATP with those obtained when elution was accomplished by NaCl gradients suggested that elution of NMN adenylyltransferase from CM-cellulose by ATP was not simply due to the contribution of the ATP to the ionic strength of the eluting buffer.

Electrophoresis on polyacrylamide gels

The patterns obtained from a pH fraction and from a CM-cellulose fraction of high specific activity upon gel electrophoresis under dissociating conditions are shown in Fig. 4. The most highly purified sample of enzyme still gave 1 major and 6 minor bands. From a consideration of the molecular weight of the native enzyme (to be presented in a subsequent section) and of the approximate molecular weights and relative concentrations of material in the various bands obtained upon electrophoresis, it is clear that not all of these can represent enzyme protein and that our most highly purified preparations are, therefore, still not homogeneous. These results also suggest that the native enzyme may consist of several polypeptide chains.

Stokes radius

The distribution coefficient (K_d) of NMN adenylyltransferase on Sepharose 6B was 0.455 (average of 8 determinations). A plot of the Stokes radii of the marker proteins versus their $(K_d)^{1/3}$ values [38] gave the expected linear relationship (Fig. 5). The Stokes radius of NMN adenylyltransferase was determined from this graph to be 62.4 Å. The distribution coefficient of the transferase remained constant in buffers containing both 0.1 M and 1.0 M NaCl, at pH 7.5 and 8.5, and in the presence and absence of ATP.

Sedimentation coefficient

Results obtained upon centrifuging NMN adenylyltransferase and the internal standards, catalase and alcohol dehydrogenase, through isokinetic sucrose gradients are presented in Fig. 6. In such gradients, the ratio of the distances traveled by any two proteins from the meniscus will always be constant, and will be equal to the ratio of the sedimentation coefficients of the proteins, assuming that they have the same partial specific volumes [39]. We used this relationship to calculate the sedimentation coefficient for NMN adenylyltransferase, using alcohol dehydrogenase as the standard. The assumption was made that both proteins had partial specific volumes ($\bar{v}$) of 0.725 cm^3 per g, and $s^{0.725}_{20,w}$ ($s_{20,w}$ calculated on the basis of $\bar{v} = 0.725$ cm^3 per g) for the dehydrogenase was taken as 7.4 [39]. An $s^{0.725}_{20,w}$ of 7.9 was obtained for the transferase (see below). As shown by Martin and Ames [39], the error in $s_{20,w}$ resulting from the assumption that the partial specific volumes are 0.725 will be small.

Molecular weight and frictional ratio

From the Stokes radius and sedimentation coefficient determined above, and assuming a partial specific volume of 0.725, a molecular weight of 203 000 and frictional ratio of 1.6 were calculated for NMN adenylyltransferase. Estimation of molecular weights and frictional ratios in this manner has been discussed by Siegel and Monty [38]; values which have been obtained using this procedure have, in general, agreed well with those determined by classical methods [38,40,41].

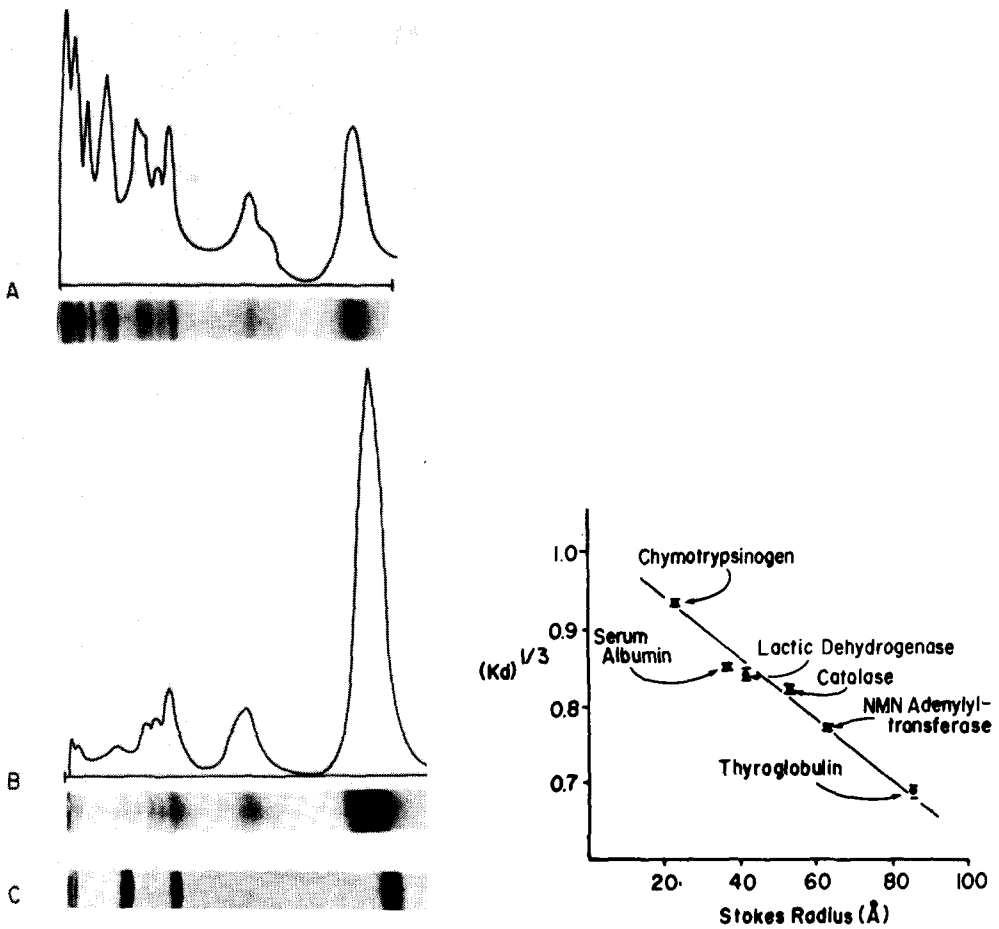

Fig. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of reduced and alkylated protein fractions. Gel and gel-scan of: A, pH fraction, specific activity 1.7; B, CM-cellulose fraction, specific activity 31. Gel C shows the pattern obtained for the molecular weight standards, from left to right, bovine serum albumin (molecular weight 68 000), chicken egg albumin (43 000), and horse cytochrome *c* (11 700). The origin of electrophoresis was at the left of the gels, with migration toward the anode. (See text for details.)

Fig. 5. Molecular size determination of NMN adenylyltransferase by gel filtration. Preparations of the transferase and proteins with known Stokes radii were chromatographed on Sepharose 6B as described in Materials and Methods. $K_d = (V_e - V_o/V_i$, where K_d is the distribution coefficient, V_e is the elution volume for the protein, V_o is the void volume taken as V_e for blue dextran (144.0), and V_i is the internal volume taken as V_e for tritiated water minus V_o (291.9 ml). Each point is the mean of several determinations ±S.E.

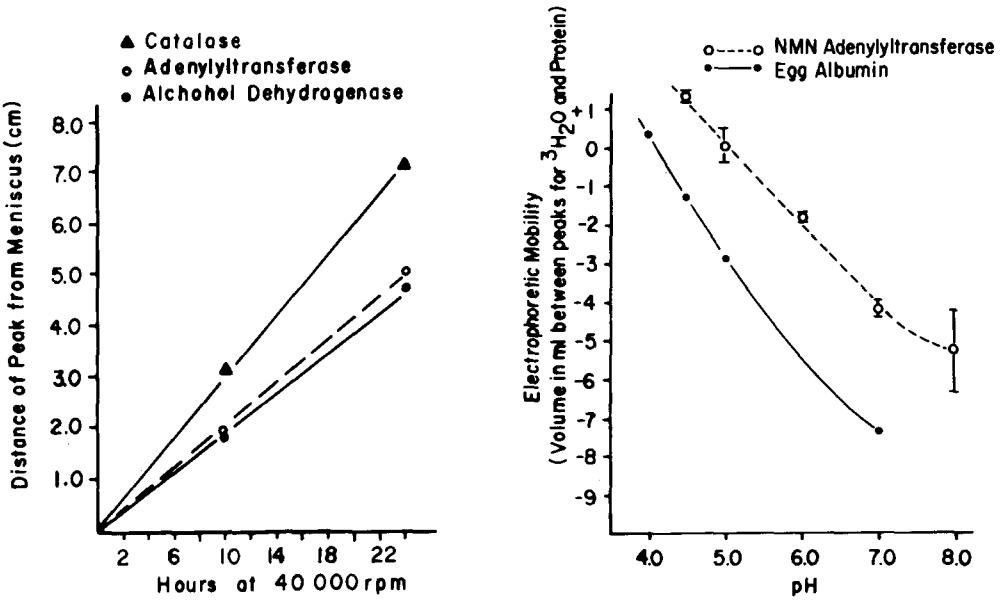

Fig. 6. Sedimentation of NMN adenylyltransferase, catalase, and alcohol dehydrogenase in isokinetic sucrose density gradients. (For details, see text.) Each enzyme gave a well-defined activity peak that covered 5—7 fractions (7 drops per fraction).

Fig. 7. pH-Electrophoretic mobility curves for NMN adenylyltransferase and egg albumin. (See text for details.) A negative mobility indicates migration to the anode. Single determinations at each pH were carried out for egg albumin. For the transferase, each point is the mean of several determinations ±S.E.

If it is assumed that the partial specific volume and degree of hydration of NMN adenylyltransferase are not unusual, then the frictional ratio obtained suggests that the molecule is appreciably asymmetric. On the assumption that the enzyme is hydrated to the extent of 0.4 g of water per g anhydrous protein, the axial ratio for a prolate ellipsoid would be approximately 7 [42].

Isoelectric point

From the pH-electrophoretic mobility curve (Fig. 7), the pI values for NMN adenylyltransferase were estimated to be 5.0. Egg albumin, run as a control, was found to have a pI of 4.1. Tiselius and Svensson [43] obtained an isoelectric point of 4.55—4.57 for this protein by moving boundary electrophoresis. Cantarow and Stollar [8] reported a pI of 5.5 for NMN adenylyltransferase from chicken erythrocytes, and Atkinson et al. [44] reported that the pI for the pig liver enzyme was close to 7. On the basis of our pI value for NMN adenylyltransferase, it may be classified as an acidic nuclear protein.

Sucrose density gradient isoelectric focusing [45] was also done on preparations of NMN adenylyltransferase. Multiple enzyme activity peaks with isoelectric points ranging from 4.5 to 8.5 were obtained, and a discrete pI could not be determined using this method.

Kinetics

Michaelis constants for ATP and NMN were determined in the presence of

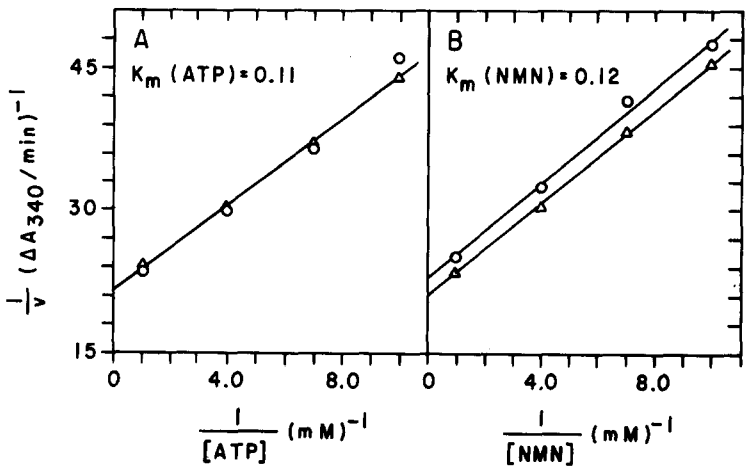

Fig. 8. Kinetic studies. A. Reciprocal velocity is plotted against reciprocal concentration of ATP at 2.25 mM (○———○) and 10.0 mM (△———△) NMN concentration. B. Reciprocal velocity is plotted against reciprocal concentration of NMN at 2.5 mM (○———○) and 9.7 mM (△———△) ATP concentration.

saturating concentrations of the second substrate (Fig. 8). The higher reaction velocities observed at the higher ATP concentration in Fig. 8 suggests that saturation had not quite been achieved at the lower ATP concentration. Our K_m value for NMN, 0.12 mM, is close to that determined by others for the pig liver enzyme using similar assay conditions and techniques: 0.15 mM [5], and 0.12 mM [46]. Our K_m value for ATP, 0.11 mM, is lower than that determined by others under similar conditions: 0.46 mM [5], and 0.4 mM [46]. However, using an assay coupled with alcohol dehydrogenase at pH 8.0, Jackson and Atkinson [47] obtained a K_m for ATP of 0.088 mM. The enzyme preparations used in the kinetic studies had no ATPase or NMNase activities.

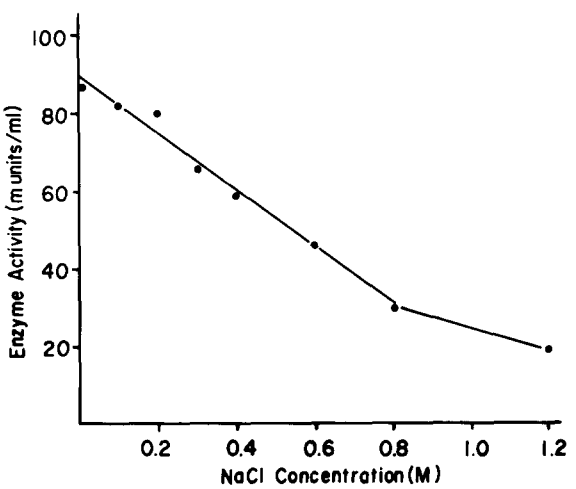

Fig. 9. Inhibition of NMN adenylyltransferase activity by NaCl. Portions of an enzyme preparation (0.28 units/ml; specific activity 0.057) were adjusted to various NaCl concentrations and the solutions assayed. The abscissa refers to the NaCl concentration in the assay reaction mixture.

Effect of ionic strength on activity

The activity of NMN adenylyltransferase is inhibited by NaCl (Fig. 9). The inhibition was reversible, activity being restored upon dialysis or dilution with buffers of low ionic strength. A similar inhibition of enzyme activity was observed with potassium phosphate.

Discussion

The purification procedure described in this paper results in a 3500-fold enrichment of NMN adenylyltransferase from extracts of pig liver nuclei (Table I). This is the most extensively the transferase has been purified from any source. Nevertheless, our most highly purified preparations were still not homogeneous, and it is apparent that the enzyme is present in very low concentrations in mammalian cells. Our observation that an enzyme preparation with a specific activity of 31 (μmol substrate converted per min per mg protein) was not homogeneous (Fig. 4) calls into question the claim [48] that crystalline pig liver NMN adenylyltransferase was obtained from an enzyme preparation with a specific activity of 0.33 [44].

Attempts to purify the enzyme using affinity chromatographic techniques were not successful. Neither an NAD^+ gel prepared by the method of Mosbach et al. [49] nor an ATP gel prepared according to Lamed et al. [50] showed any specific affinity for the transferase.

One of the very interesting aspects of NMN adenylyltransferase is its intranuclear localization. Among the questions which this raises are: (1) What keeps the enzyme from moving out into the cytoplasm? (2) In view of the fact that the oxidation-reduction reactions which require NAD^+ as a coenzyme occur predominantly in the extranuclear compartments of the cell, what is the selective advantage of having the enzyme which synthesizes NAD^+ in the nucleus? Some of our studies on the properties of the enzyme bear on the first of these questions. Since our data indicate an isoelectric point of about 5 for NMN adenylyltransferase, it cannot, like the histones, be bound to the nuclear DNA by simple ionic interactions. If the enzyme is retained in the nucleus as a result of binding to some nuclear constituent, this binding must be of a more specific nature. However, binding to a nuclear component may not be required to explain the retention of the enzyme in the nucleus, for there is evidence that proteins of high molecular weight do not pass across the nuclear envelope, at least in some systems [51,52]. Our finding that NMN-adenylyltransferase has a molecular weight of about 200 000 suggests that molecules of this enzyme, once inside the nucleus, may not be able to exit from it at a significant rate.

The selective advantage which the intranuclear localization of NMN adenylyltransferase confers on the cell is not readily apparent. However, observations from several laboratories may be related to this question: (1) Eukaryotic cell nuclei contain enzyme systems which convert NAD^+ to poly (ADP-ribose) [53], which is thought to serve a structural [54] or regulatory [55] role within the nucleus. (2) NMN adenylyltransferase activity has been correlated with DNA synthesis [12,17—21] suggesting that NAD^+, in addition to serving as a coenzyme, may play a role in DNA replication. Future studies on these problems will be facilitated by a method for obtaining the mammalian

transferase in a highly purified state. A source of purified enzyme should also prove useful to those wishing to synthesize NAD^+ labeled in various positions.

Acknowledgments

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