Nicotinamide Mononucleotide Adenylyltransferase. Molecular and Enzymatic Properties of the Homogeneous Enzyme from Baker's Yeast†

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ABSTRACT: Nicotinamide mononucleotide (NMN) adenylyltransferase has been purified to homogeneity from baker's yeast crude extract. The purification procedure is relatively simple and consists of high-salt extraction of enzyme activity and precipitation with poly(ethylenimine), followed by ion-exchange and dye ligand chromatography separations. The final enzyme preparation is homogeneous as judged by a single Coomassie blue stainable band when run on nondenaturating and denaturating polyacrylamide gels. The native enzyme shows a molecular weight of about 200 000, calculated by gel filtration and sucrose gradient centrifugation. The protein possesses quaternary structure and is composed of four apparently identical M_r 50 000 subunits. The absorption spectrum shows a maximum at 280 nm and a minimum at 253 nm. The isoelectric point is 6.2. Amino acid composition analysis shows the presence of 28 half-cystine residues. The same result has been obtained by titrating the enzyme in denaturating conditions with Ellman's reagent after incubation with sodium borohydride. NMN adenylyltransferase is a glycoprotein containing 2% sugar, 2 mol of alkali-labile phosphate per mole of enzyme, and 1 mol of adenine moiety per mole of enzyme. Therefore, the possibility that the enzyme is ADP-ribosylated exists. The K_m values for ATP, NMN, and nicotinate mononucleotide are 0.11 mM, 0.19 nM, and 5 mM, respectively. Kinetic analysis reveals a behavior that is consistent with an ordered sequential Bi-Bi mechanism. The pH optimum is in the range 7.2–8.4.

Since the discovery that NMN[1] adenylyltransferase (ATP:NMN adenylyltransferase; EC 2.7.7.1) is exclusively located in the cell nucleus (Hogeboom & Schneider, 1952; Chambon et al., 1966; Atkinson et al., 1961; Ferro & Kuehl, 1973; Siebert & Humphrey, 1965), various authors have suggested that NAD fulfills another role in addition to that of respiratory coenzyme in eukaryotic cells. Morton (1958) hypothesized that NAD might have a key role in the regulation of cell proliferation in that the nucleus may sense changes in intracellular NAD concentrations. Thereafter, the discovery that another nuclear enzyme, poly(ADP-ribose) synthetase, utilizes NAD as a substrate for the modification of nuclear proteins (Chambon et al., 1966) suggested that this enzyme may be involved in the mechanism by which NAD concentrations are sensed by the nucleus (Purnell et al., 1980). In prokaryotes, NAD is known to be a substrate in the DNA ligase catalyzed joining of DNA fragments (Olivera et al., 1968). Both ADP ribosylation and DNA ligase reactions result in the breakdown of the NAD molecule, yielding free nicotinamide and nicotinamide mononucleotide, respectively, which are then recycled back to the dinucleotide through appropriate salvage pathways, known as "pyridine nucleotide cycles" (Hillyard et al., 1981; Gholson, 1966). At least two distinct pathways have been recognized in prokaryotes (pathway 1) and eukaryotes (pathway 2).

$$\text{NAD} \rightarrow \text{NMN} \rightarrow \text{NaMN} \rightarrow \text{NaAD} \rightarrow \text{NAD} \quad (1)$$

$$\text{NAD} \rightarrow \text{nicotinamide} \rightarrow \text{NMN} \rightarrow \text{NAD} \quad (2)$$

The occurrence of these reaction cycles results in surprisingly high turnover rates for the NAD molecule, with half-lives in the order of 2 h in *Escherichia coli* and rat liver, 1 h in HeLa D98/AH2 cells, and 25 min in *Physarum polycephalum* (Hillyard et al., 1981). Besides, NAD turnover is highly suppressed in enucleated cells (Rechsteiner & Catanzarite, 1974), where the NAD half-life increases up to 10 h, so that nuclear events seem to be responsible for both breakdown and resynthesis of NAD. While the biological significance of NAD turnover taking place at the nuclear level is as yet unclear, it seems of fundamental importance to study the individual enzymes involved in this phenomenon. A central role in NAD salvage synthesis is played by NMN adenylyltransferase.

In partially purified preparations, the same enzyme has been shown to catalyze the formation of the dinucleotide in both pathways 1 and 2 without specificity toward NMN or deamido-NMN (Dahmen et al., 1967; Cantarow & Stollar, 1977). This enzyme, first described by Kornberg (1948), reversibly catalyzes the synthesis of NAD from nicotinamide mononucleotide and ATP with the concomitant formation of pyrophosphate.

Unfortunately, no studies on the homogeneous enzyme have so far been reported, owing principally to difficulties in the purification procedure. In our laboratory we have found that the yeast enzyme is almost completely associated with the chromatin fraction (Ruggieri & Magni, 1982). The present paper deals with the purification procedure and the major molecular and enzymatic properties of the homogeneous enzyme from yeast.

Experimental Procedures

Materials

NMN, deamido-NMN (NaMN), NAD, AMP, ADP-ribose, and ATP were obtained from Sigma, [8-^{14}C]ATP was

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[1] Abbreviations: NMN, nicotinamide mononucleotide; NaMN, nicotinate mononucleotide; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); Hepes, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; PP_i, inorganic pyrophosphate; NAD, nicotinamide adenine dinucleotide; DTT, dithiothreitol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

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from Amersham, poly(ethylenimine), 50% solution, was from Serva, and poly(ethylenimine)–cellulose (PEI-F) and Whatman 3MM thin-layer sheets (with fluorescent indicator) were from J. T. Baker and Eastman Kodak, respectively. DE-52 cellulose was from Whatman. Matrex Gel Green A was from Amicon Corp. Ampholine carrier ampholytes were obtained from LKB. Enzymes and protein markers were from Sigma or Boehringer Mannheim. All other chemicals were of the best available grade.

Methods

Preparation of Cell Extracts. Cell extracts were prepared from commmercial compressed yeast (Vulcania, Italy) by homogenization through a French press. All steps were performed at 0–4 °C. Cells (200 g wet weight) were suspended in 200 mL of 0.2 M potassium phosphate buffer, pH 7.4, containing 1 mM DTT and 5 mM $MgCl_2$, and passed through a French pressure cell at 2000 k/cm^2. This operation was repeated, with cooling intervals, while the appearance of the homogenate was checked by phase contrast microscopy. Usually two passes were sufficient to break more than 80% of the cells. One more pass was made to achieve extensive shearing of chromosomal material.

Assay of Enzyme Activity. Enzyme activity was routinely tested by a continuous spectrophotometric coupled enzyme assay modified after Kornberg (1950). The reaction mixture contained, in a final volume of 0.850 mL, 60 mM Hepes buffer, pH 7.6, 1.18 mM NMN, 1.47 mM ATP, 20.7 mM $MgCl_2$, 35 mM semicarbazide hydrochloride, 0.45% ethanol, 7.8 units of yeast alcohol dehydrogenase, 0.59 mg/mL bovine serum albumin, and the appropriate amount of sample to be assayed. The reaction was started by addition of NMN and continuously followed at 340 nm in a Cary 118C spectrophotometer. The temperature was maintained at 37 °C. Alternatively, the activity was assayed by measuring the radioactivity incorporated into NAD from labeled ATP after chromatographic or electrophoretic separation. The reaction mixture contained, in a final volume of 100 μL, 2.25 mM [8-^{14}C]ATP (5.8 mCi/mmol), 2.5 mM NMN, 50 mM Tris-HCl, pH 7.6, 20 mM $MgCl_2$, and the appropriate amount of enzyme. After a 30-min incubation at 37 °C the reaction was terminated by addition of 10 μL of 40% (w/v) trichloroacetic acid. After centrifugation, 10 μl of supernatant was spotted on a cellulose PEI-F thin layer together with an appropriate amount of cold NAD and ATP and chromatographed with 1 M acetic acid as the solvent. After the chromatogram was developed and dried, the spots corresponding to NAD and ATP were cut out and placed in vials with 12 mL of scintillation mixture [4 g of 2,5-diphenyloxazole (PPO) and 0.05 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) per liter of toluene] for counting in a Philips PW4540 liquid scintillation spectrometer. When the synthesis of deamido-NAD from deamido-NMN was measured, the nucleotides were separated by electrophoresis essentially as described by Atkinson et al. (1961) and counted as described above.

Polyacrylamide Gel Electrophoresis. Discontinuous polyacrylamide gel electrophoresis was conducted according to Ornstein (1964) and Davis (1964) in 5% gels. Approximately 0.06 unit of enzyme was loaded and run at 5 mA per gel. After electrophoresis gels were either stained for protein with Coomassie brilliant blue R-250 or sliced into 2-mm sections to test for enzyme activity. Slices were incubated overnight with 250 μL of 100 mM Hepes buffer, pH 7.6, containing 40 mM $MgCl_2$. Aliquots were then assayed for enzyme activity by a standard spectrophotometric assay. Alternatively, gels were stained for glycoproein with the periodic acid–Schiff (PAS) stain according to Matthieu and Quarles (1973).

Polyacrylamide Gel Electrophoresis (Denaturating). For estimation of subunit molecular weight, 20-μg aliquots of pure NMN adenylyltransferase were incubated at 100 °C for 5 min in a denaturating mixture containing 2% SDS, 5% 2-mercaptoethanol, and 0.125 M Tris-HCl, pH 6.8. Samples were applied to 8-cm 10% polyacrylamide gels and run at 5 mA per gel essentially as described by Laemmli (1970).

Isoelectrofocusing. Isoelectrofocusing was conducted on a 110-mL LKB 8100 ampholine column filled with a linear, 0–560 g/L, gradient of sucrose containing 2% (w/v), pH 3.5–10, ampholine carrier ampholytes either in the presence or in the absence of 4 M urea. Aliquots of pure enzyme, containing approximately 5 units of activity, were added to the dense sucrose solution before pouring the gradient. Runs were conducted at 4 °C and 15-W constant power for 18 h. The column was eluted at a flow rate of 60 mL/h; 1.0-mL fractions were collected. Fractions were tested for pH and enzyme activity.

Analysis of Total and Alkali-Labile Phosphate. Analysis of phosphate content of pure NMN adenylyltransferase was performed essentially as described by Martensen (1984), by using phosphorylase *b* and phosphorylase *a* as the controls.

Determination of Adenine Derivatives. Adenine derivatives were estimated by reaction with glyoxal hydrate trimer according to Yuki et al. (1972), using AMP and ADP-ribose as the standards.

Carbohydrate Analysis. Carbohydrate content was estimated by the phenol–sulfuric acid procedure of Dubois et al. (1956). Glucose was used as the standard and ovalbumin as a control glycoprotein, assuming a 3.4% sugar content.

Analysis of Sulfhydryl Groups. Sulfhydryl group analysis was performed by using Ellman's reagent, DTNB, in the presence of guanidine as denaturating agent, according to the procedure described by Habeeb (1973).

Amino Acid Analysis. For amino acid analysis, duplicate samples of protein (0.1 mg) were hydrolyzed for 24, 36, and 48 h at 120 °C in 6 N HCl and 0.1% phenol under vacuum; analysis was performed on an LKB Model 3201 amino acid analyzer. Tryptophan was measured according to Bencze and Scmid (1957). Half-cystine residues were determined as described by Spencer and Wold (1969).

Molecular Weight. The molecular weight of the enzyme was determined both by gel filtration (Andrews, 1969) and by sucrose gradient ultracentrifugation according to Martin and Ames (1961). The gel filtration experiment was performed on a 1.6 × 55.5 cm Sephadex G-200 superfine column equilibrated with the eluted by 50 mM Tris-HCl buffer containing 1 mM DTT, 100 mM KCl, and 0.2 mg/mL bovine serum albumin, pH 7.2. The column was calibrated with the following markers of Known molecular weight: rabbit muscle pyruvate kinase (M_r 240 000), hemoglobin (M_r 67 000), phosphorylase *b* (M_r 92 000), and chymotrypsinogen (M_r 25 000). In a separate run, 0.26 unit of pure enzyme was applied to the column. The column was eluted at a flow rate of 10 mL/h, and 0.5-mL fractions were collected. For sucrose gradient ultracentrifugation, Beckman SW50.1 centrifuge tubes were loaded with a 5–50% sucrose gradient in 50 mM Tris-HCl buffer, pH 7.5, and stored at 3 °C for 12 h before use. The following markers of known molecular weight were used: beef liver catalase (M_r 250 000), yeast alcohol dehydrogenase (M_r 150 000), and lysozyme from chicken egg white (M_r 17 200). The centrifugation was conducted at 190000*g* for 18 h at 3 °C. Gradients were then eluted by piercing the tube bottoms with a syringe needle and collecting

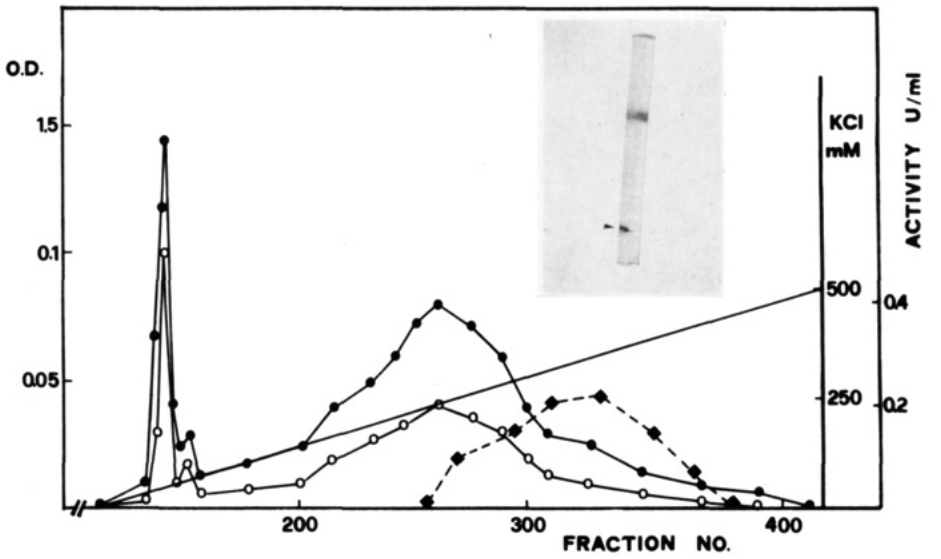

FIGURE 1: Matrex Gel Green A dye ligand chromatography. The enzyme activity was eluted with a linear gradient, 0–500 mM KCl in 20 mM Tris-HCl buffer, pH 7.9 (continuous line); 1-mL fractions were collected. Symbols: (●) optical density at 280 nm; (○) optical density at 260 nm; (◆) enzyme activity. The inset shows the disc gel electrophoresis of a 20-μg sample of pooled fractions on 5% polyacrylamide gel according to Davis as described under Methods. Arrow indicates the position of the dye.

2-drop fractions with an automatic fraction collector. A constant flow rate was maintained by pumping air into the top of the tubes with a peristaltic pump, through an airtight rubber connection. Fractions were tested either for enzyme activity by the appropriate enzyme assay according to the Biochemica Information Handbook or for protein content.

Protein Determination. Protein content was measured as described by Bradford (1976) using bovine serum albumin as the standard.

Results and Discussion

Purification of the Enzyme. Total cell homogenate, obtained from 200 g of yeast cells as described under Methods, was made 3 M in KCl by adding solid salt. After being stirred for 2 h in the cold, the suspension was centrifuged for 40 min at 25000*g* and the supernatant extensively dialyzed against 25 mM potassium phosphate, pH 7.4. It should be pointed out that by omitting high-salt extraction, only about one-third of the activity was obtained. A 3 M KCl concentration was found by separate experiment to give maximal yield of enzyme activity with minimal detrimental effect on the specific activity. To the dialyzed KCl extract, solid ammonium sulfate was added up to 50% saturation. The pH was maintained at 7.4 by dropwise addition of 1 M NH_4OH. After being stirred for 30 min in the cold, the precipitated protein was collected by centrifuging for 40 min at 25000*g* and carefully resuspended with the aid of a Potter homogenizer in 75 mL of 20% saturated ammonium sulfate in 25 mM potassium phosphate buffer containing 1 mM DTT, pH 7.4. The resulting suspension was centrifuged for 30 min at 25000*g* and the 20% saturated ammonium sulfate washing repeated on the collected precipitate. The combined supernatants were dialyzed extensively against 10 mM potassium phosphate buffer, pH 7.4, containing 1 mM DTT. The dialyzed ammonium sulfate fraction exhibited a A_{280}/A_{260} ratio of 0.88, revealing contamination with 260 nm absorbing material. Such material could be removed by precipitation with 0.3% poly(ethylenimine). To the 20–50% ammonium sulfate fraction was added dropwise a 10% (v/v) solution of poly(ethylenimine) (pH 7.4), while the mixture was stirred on ice, to a final concentration of 0.3%. After the mixture was stirred for 20 min, the precipitate was collected by centrifugation at 12000*g* for 10 min, carefully resuspended with a Teflon pestle homogenizer in 50 mM Tris-HCl containing 0.1 mM EDTA, 1 mM DTT, and 200 mM ammonium sulfate, pH 7.4, stirred for 20 min, and centrifuged at 12000*g* for 20 min.

The supernatant was made 60% saturated by the addition of solid ammonium sulfate. After standing for 20 min in the cold, the suspension was centrifuged for 20 min at 12000*g*. The pellet was dissolved in the original volume of 10 mM potassium phosphate buffer, pH 7.4, and extensively dialyzed against the same buffer.

This step yielded 90% recovery of enzyme activity and only 19% recovery of protein, resulting in about a 5-fold increase in specific activity. Removal of most of the nucleic acids was also achieved, as shown by the increase of the A_{280}/A_{260} ratio from 0.88 to 1.65. Sixty percent ammonium sulfate precipitation of proteins was necessary in order to remove residual poly(ethylenimine). The dialyzed material was loaded onto a DE-52 (5 × 10 cm) column equilibrated with the above buffer. The column was washed with the starting buffer to remove unbound material, as revealed by monitoring absorbance at 280 and 260 nm. Then a linear gradient from 10 to 300 mM potassium phosphate, pH 7.4, was applied, followed by 1 M buffer washing. Most of the activity was eluted as a single peak at about 100 mM buffer concentration. The A_{280}/A_{260} ratio was higher than 1 throughout the elution profile. It should be stressed that by omitting the PEI precipitation step the bulk of the activity was strongly retained on DEAE-cellulose and could only be eluted at 1 M buffer. Active fractions were pooled, concentrated through an Amicon PM-10 membrane, and dialyzed against 20 mM Tris-HCl buffer, pH 7.9. The dialyzed pool was applied to a 3 × 5 cm Matrex Gel Green A dye ligand chromatography column, equilibrated with the above buffer. The elution was performed with a linear gradient, 0–500 mM KCl in 20 mM Tris-HCl buffer, pH 7.9. The activity was eluted at a 250–400 mM KCl concentration (Figure 1). Aliquots of the active fractions were individually subjected to polyacrylamide gel electrophoresis to check for their homogeneity after dialysis against 20 mM Hepes, pH 7.5. Homogeneous fractions were pooled and dialyzed against 20 mM Hepes, pH 7.5. A sample of this material was analyzed on polyacrylamide gel electrophoresis. A single band was shown by Coomassie staining (Figure 1,

Table I: Purification of Yeast NMN Adenylyltransferase

step	total protein (mg)	total act. (units)	sp act. (units/mg)	purification (x-fold)	yield (%)
KCl extract	36625	35.6	0.00098		100
ammonium sulfate	14650	28.5	0.0019	1.9	80
poly(ethylenimine)	2100	20.4	0.0097	9.9	57.3
DE-52	246	18.1	0.073	74.5	50.8
Matrex Gel Green A	4.3	12.1	2.8	2857	34

[a] Activity at this step was measured by radioactive assay (see Methods), to overcome interferences in the spectrophotometric assay. In subsequent steps spectrophotometric assay was used.

inset), whose relative migration (0.44) corresponded to that of the activity assayed on 2-mm slices of the gel as described under Methods. The purification procedure is summarized in Table I.

Stability. After 2 weeks at 4 °C, the pure enzyme retained 70% of its original activity in 20 mM Hepes buffer, pH 7.5. After 1 month at −20 °C, the activity was 80%, in the same buffer.

Absorption Spectrum. The absorption spectrum of pure enzyme showed a maximum at 280 nm and a minimum at 253 nm. The A_{280}/A_{260} ratio was 1.86. The percent absorption coefficient E was 4.91.

Molecular Weight. The molecular weight of the native enzyme estimated by gel filtration and sucrose gradient ultracentrifugation, as described under Methods, was 200 000 ± 10 000 and 200 000 ± 5000, respectively. These values are in agreement with those reported by other authors for the enzyme from other sources (Cantarow & Stollar, 1977; Kono et al., 1978; Ferro & Kuehl, 1975; Adamietz et al., 1979). The gel filtration experiment was conducted with 0.2% bovine serum albumin in the buffer. In the absence of bovine serum albumin less than 1% activity could be recovered. The protective action exerted by bovine serum albumin is under investigation. A single band was observed upon sodium dodecyl sulfate–polyacrylamide gel electrophoresis, of M_r 50 000 ± 1000. Identical behavior was shown both in the presence and in the absence of 2-mercaptoethanol. Therefore, the enzyme appears to be composed of four electrophoretically indistinguishable subunits not linked by disulfide bridges.

Isoelectric Point. The isoelectric point was at pH 6.2 in the presence of 4 M urea. In the absence of urea, multiple p*I*'s were observed in the acidic range, namely, at pH 4.2, 4.9, and 6.2, with a total peak activity ratio of approximately 1:1:3 (Figure 2). This behavior is analogous to that observed for the chicken enzyme (Cantarow & Stollar, 1977) and can be interpreted as the result of aggregation of the enzyme molecules as observed for other nuclear proteins (Adamietz et al., 1979).

Amino Acid Composition. Amino acid analysis (Table II) showed a relative excess of the acidic amino acid residues aspartic acid and glutamic acid. In addition, the enzyme contained 7 half-cystine residues per subunit (M_r 50 000).

Sulfhydryl Group Content. Analysis of sulfhydryl group content with DTNB, performed with and without previous reduction with sodium borohydride, revealed in both cases the presence of 28 mol of sulfhydryl groups per mole of enzyme (7 per subunit), indicating the absence of disulfide bridges.

Carbohydrate and Phosphate Content. The enzyme was shown to be a glycoprotein by periodic acid–Schiff staining, following polyacrylamide gel electrophoresis. In order to measure sugar content, an aliquot of pure enzyme was analyzed by the phenol–sulfuric acid method (Dubois et al., 1956) after hydrolysis in 2 N H_2SO_4, and the results indicated that the preparation contained about 2% carbohydrate by weight, using for ovalbumin taken as a standard. Total phosphate and alkali-labile phosphate analyses were also conducted on pure

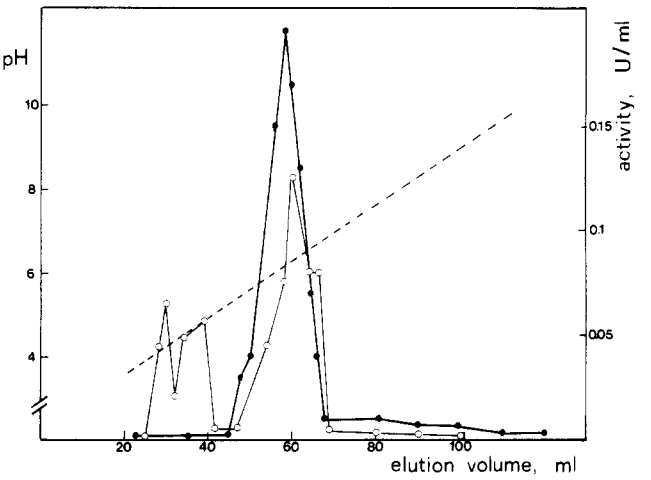

FIGURE 2: Isoelectrofocusing of NMN adenylyltransferase. The experiment was performed as described under Methods by using 5 units of enzyme activity from the last step of the purification procedure, in the presence (●) and in the absence of (○) of 4 M urea. The dashed line represents the pH gradient profile.

Table II: Amino Acid Composition of Yeast NMN Adenylyltransferase[a]

amino acid	residues per subunit	amino acid	residues per subunit
Asp and Asn	35	Val	35
Glu and Gln	52	Phe	16
Ser	35	Ile	23
His	4	Leu	26
Gly	39	Lys	25
Thr	10	Pro	12
Arg	19	$^1/_2$-Cys[d]	7
Ala	29	Trp	5
Tyr	17	total	396
Met	7		

[a] All calculations were based on a molecular weight of 50 000. Figures represent nearest integer and are the average from five different preparations. [b] Values were extrapolated to zero time of hydrolysis. [c] Values were extrapolated to infinite time of hydrolysis. [d] Determined as cysteic acid after dimethyl sulfoxide oxidation.

NMN adenylyltransferase by using the ammonium molybdeate–malachite green procedure (Martensen, 1984). Two moles of total phosphate was detected per mole (M_r 200 000) of enzyme. The same result was obtained when alkali-labile phosphate was analyzed.

Adenine Derivatives Content. Pure enzyme, 100 μg, was analyzed for the presence of adenine derivatives by using glyoxal hydrate trimer according to Yuki et al. (1972). The results show the presence of 0.5 nmol of adenine derivatives, i.e., 1 mol per mole of enzyme.

pH Optimum. The enzyme activity was optimal in the range between pH 7.2 and pH 8.4, as determined by using an overlapping buffer system containing 20 mM each of acetic acid, succinic acid, glycylglycine, and glycine adjusted to a suitable pH with KOH. The activity was determined by ra-

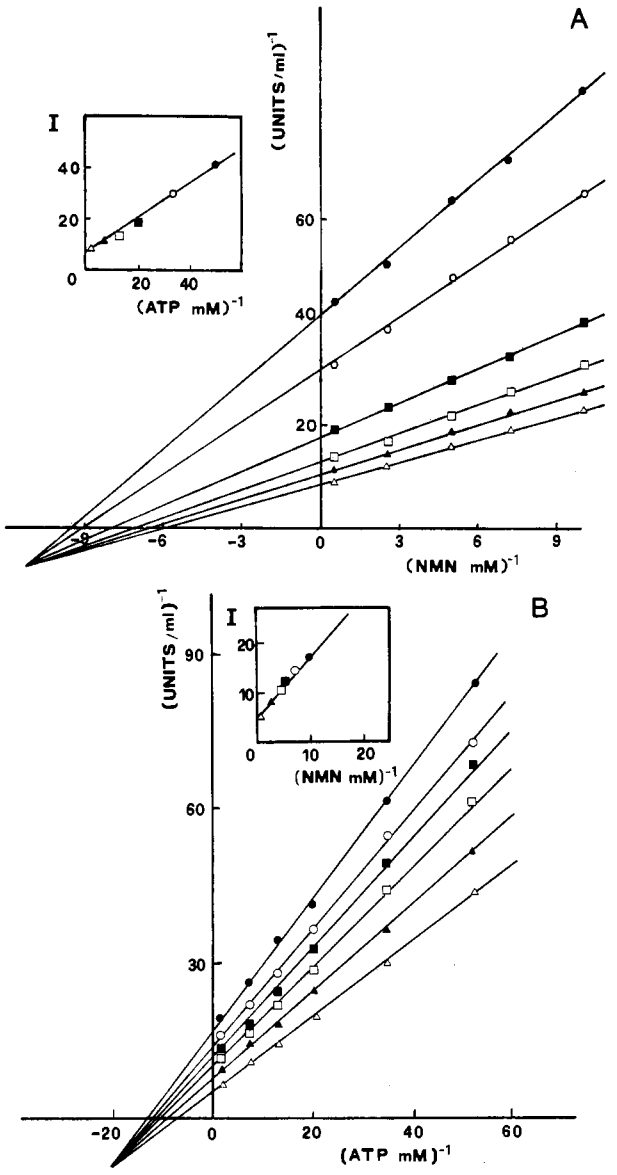

FIGURE 3: Kinetics of initial velocity of baker's yeast NMN adenylyltransferase. (A) NMN at different concentrations (in the range 0.1–2 mM) and ATP at concentrations of (●) 0.02, (○) 0.03, (■) 0.05, (□) 0.08, (▲) 0.14, and (△) 0.70 mM. The inset represents the secondary plot with the reciprocal of ATP concentrations in the abscissa and the relative intercepts in the ordinate. (B) ATP at different concentrations (in the range 0.02–0.6 mM) and NMN at concentrations of (●) 0.01, (○) 0.15, (■) 0.2, (□) 0.25 (▲) 0.4, and (△) 2.0 mM. The inset represents the secondary plot with the reciprocal of NMN concentrations in the abscissa and the relative intercepts in the ordinate.

dioactive assay (see Methods).

Enzyme Kinetics. NMN adenylyltransferase exhibits linear kinetics with respect to both NMN and ATP, having K_m values of 0.19 mM and 0.11 mM, respectively (Figure 3), as calculated from the intercept replots shown in the insets. A similar pattern was obtained when NaMN was used as the substrate. The K_m value was 5 mM. The enzyme reaction was inhibited by pyrophosphate. Double-reciprocal plots showed linear noncompetitive inhibition by this product with respect to both ATP and NMN. The K_i was 3.5 mM. Such kinetic behavior is consistent with an ordered Bi-Bi mechanism for the forward reaction, according to Cleland's nomenclature, even though more detailed product inhibition experiments need to be done before a final conclusion can be drawn. The observation that the K_m for NaMN is 1 order of magnitude higher than that for NMN suggests that the amido pathway is predominant in yeast.

Registry No. ATP, 56-65-5; NMN, 1094-61-7; NaMN, 321-02-8; NMN adenylyltransferase, 9032-70-6.

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