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Studies on the *de novo* Biosynthesis of NAD in *Escherichia coli*

The Separation of the *nadB* Gene Product from the *nadA* Gene Product and Its Purification

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Quinolinic acid (pyridine 2,3-dicarboxylic acid) which is an immediate precursor of the pyridine nucleotides, is synthesised from L-aspartate and dihydroxyacetone phosphate in *Escherichia coli*. Extracts from certain *nadB* mutants complement the extracts prepared from all *nadA* mutants for the enzymic synthesis of quinolinate. Using the complementation assay, the quinolinate synthetase B protein has been purified more than 300-fold. The quinolinate synthetase B protein exists in all *nadA* and *nadC* mutants examined. The quinolinate synthetase A protein was present in all *nadC* mutants and most (but not all) *nadB* mutants. The facile separation of the wild-type quinolinate synthetase A and B proteins out of a *nadC* mutant suggests that quinolinate synthetase does not exist as a tightly bound complex. The partially purified quinolinate synthetase is inhibited by physiological concentrations of NAD and NADH but not by NADP or NADPH.

The pyridine nucleotides, nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP) are present in all living cells and serve as substrates for an unusually large and diverse number of cellular oxidative-reductive reactions. The biosynthetic sequence which produces the pyridine nucleotides in bacteria has not been characterized, although the tryptophan to quinolinic acid to NAD pathway is known to exist in mammals and fungi [1,2]. In prototrophic bacteria and plants, quinolinic acid is also an immediate precursor of the pyridine nucleotides [3,4] but the biosynthetic pathways from L-aspartic acid to quinolinate remain to be elucidated [5,6]. In aerobic species, the seven carbon atoms of quinolinic acid are derived from dihydroxyacetone phosphate and L-aspartate, while anaerobic species derive the carbon skeleton from *N*-formyl-L-aspartate and acetyl-coenzyme A [7–9].

The enzymic synthesis of quinolinic acid in both wild-type and certain nicotinic-acid-requiring (nad^-)-strains of *Escherichia coli* has been described in cell-free extracts [6,8].

The biochemical genetics of strains of *E. coli* requiring nicotinic acid (or nicotinamide), which are referred to as *nad* mutants, have been extensively investigated. The *nadA*, *nadB* and *nadC* loci have been mapped by transduction and are located at approximately 17, 49 and 1.5 min on the *E. coli* chromosome, respectively [10,11]. The *nadC* mutants lack significant amounts of the enzyme quinolinate phosphoribosyltransferase (decarboxylating), excrete excessive amounts of quinolinate into the growth medium and contain the enzyme(s) required for the synthesis of quinolinate from L-aspartate and triose phosphate [5,6,8]. These results support the hypothesis that the *nadC* locus codes for the structural gene for quinolinate phosphoribosyltransferase. The role of the *nadA* and *nadB* gene products remains to be clarified. These mutants will utilize quinolinate for growth, contain the enzyme quinolinate phosphoribosyltransferase and do not synthesize quinolinate *in vitro*, which suggests that *nadA* and *nadB* loci code for proteins in the quinolinate biosynthetic pathway [7,12]. The *nadA* and *nadB* gene products can be complementary, since quinolinate synthesis is observed when any *nadA* extract is added to certain *nadB* ex-

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Enzyme. Quinolinate phosphoribosyltransferase (decarboxylating) (EC 2.4.2.19).

Trivial names. Quinolinic acid, pyridine 2,3-dicarboxylic acid; Bicine, *N*,*N*-bis(2-hydroxyethyl)-glycine.

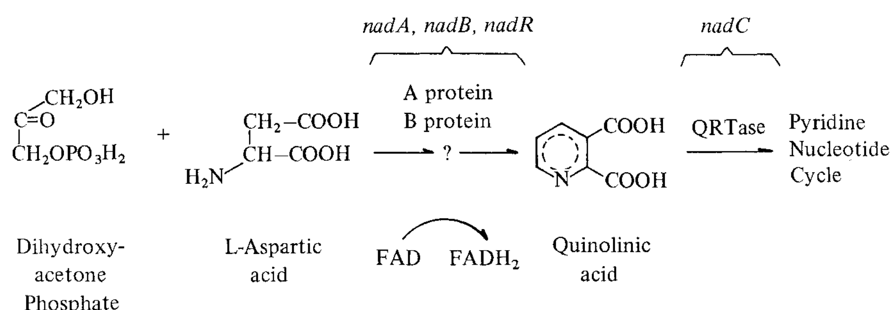

Fig. 1. *The quinolinate synthetase system of* Escherichia coli. QRTase = quinolinate phosphoribosyltransferase

tracts, under the appropriate conditions [12]. However, other *nadB* extracts fail to complement with either any *nadA* extract or any *nadB* extract. No evidence for the accumulation of any intermediates between L-aspartate and quinolinate has been obtained in any *nad* mutant extract [7,12].

The observations could suggest that the complete reaction sequence from L-aspartate to quinolinate occurs on an enzyme complex composed of the *nadA* and *nadB* gene products. The non-complementary *nadB* mutants could then be explained by postulating that the mutant *nadB* gene product is produced in excess and is virtually irreversibly bound to the *nadA* gene product. Alternatively, the non-complementary *nadB* mutants, which have been referred to as *nadR* [12], may be regulatory mutants which fail to produce either the *nadA* protein or the *nadB* protein. These interrelationships are summarized in Fig. 1.

In this paper we reported the purification of quinolinate synthetase B protein and its facile separation from the quinolinate synthetase A protein in extracts of *nadC* mutant.

MATERIALS AND METHODS

Bacterial Strains

The bacterial strains used are listed in Table 1.

Table 1. *Bacterial strains*

Strain number	Genotype	Source
PA-2-18	*nadA19, argA, thi, strA, xyl, mtl, mal, lac, gal*	R. A. La Valle
AT-11-23	*nadA28, thr, leu*	R. A. La Valle
SB-16	*nadB15, met*	R. A. La Valle
W-3899	*nadB30*	T. K. Sundarum
E-18	*nadR, thi, mtl, xyl, ara, gal, lac*	E. A. Adelberg
E-126	*nadC13*	J. Imsande
SB-99	*nadC27, met*	R. A. La Valle
W-4516	*nadC32*	T. K. Sundarum

Growth Conditions

The minimal media of Yates and Pardee [13] supplemented with 0.5 μM nicotinic acid and other necessary growth factors were used. After harvesting the bacteria on a Sharples centrifuge, the cell paste was washed with saline and stored at −20 °C until used. The enzyme activity remained stable in the cells for more than 4 months.

Preparation of Crude Extracts

The crude extract of the *E. coli* K-12 PA-2-18 used for enzyme purification was prepared by suspension of 60 g of frozen cells in 300 ml of 0.05 M Bicine buffer, pH 8.0. When a homogeneous suspension was obtained, a volume of 350 ml was sonicated for 18 min at 0−4 °C in a large rosette cell with a Branson sonifier set at 10 A (D.C.). The sonicate was centrifuged at 40000 × *g* for 45 min to remove cell debris, yielding about 325 ml of crude extract.

Preparation of A Protein for Assay of the B Protein

In order to assay for the B protein it was necessary to prepare the relatively unstable A protein immediately prior to the assay. The A protein for the quinolinic acid synthesis assay was partially purified by heat treatment and salt fractionation from strain SB-16 before use in the assay. The crude extract, prepared as described above, was mixed for 5 min in a 55 °C water bath and subsequently cooled 10 min in a water-ice bath. After centrifugation for 15 min at 20000 × *g*, about 50 ml of heat-treated supernatant were obtained. Solid ammonium sulfate was added gradually over 15 min to this supernatant and stirred at 0−4 °C to reach 40% saturation of ammonium sulfate. After mixing for 30 min, the solution was centrifuged at 20000 × *g* for 15 min. The supernatant was treated with ammonium sulfate in the same manner to bring it to 60% saturation. After 30 min

mixing and 15 min centrifugation at 20000 × g, the supernatant was discarded and the pellet was redissolved in 0.005 M potassium phosphate buffer, pH 7.5, containing 0.05 M KCl (0.2 ml/mg pellet). These steps result in a 7-fold final increase in the specific activity of the A protein over the crude extract.

Enzyme Assay for Quinolinic Acid Synthesis

Measurements of the enzymic activities of the A and B proteins were made using the procedures described by Suzuki *et al.* [7] and Chandler and Gholson [14] with some modifications.

Reaction mixtures were prepared as follows: each assay sample contained 0.25 µmol of L-aspartate, 1 µmol of fructose 1,6-bisphosphate or dihydroxyacetone phosphate, 0.01 µmol FAD, and 30 µmol of Bicine buffer, pH 8.0 (adjusted with KOH) in a volume of 0.1 ml. Also 0.1 ml of a 5-µCi/ml [U-^{14}C]aspartic acid solution (specific activity 160 mCi/mmol) was added to each tube. Enzyme preparations totaling 0.3 ml were added, making a final volume in the assay tubes of 0.5 ml.

Assay of the B protein used an excess of the A protein such that the activity of the B protein limited the rate of quinolinic acid synthesis. Routinely, 0.2 ml of the 40 – 60% ammonium sulfate fraction from strain SB-16 and 0.1 ml of diluted B enzyme fraction were added to each assay tube. These volumes represent 0.3 – 0.5 mg and 0.01 – 3 mg protein, respectively. When activity of the A protein was measured, B protein was added in excess as 0.1 ml of the 0 – 50% sodium citrate fraction and the A protein was added in a limiting amount. The use of fructose 1,6-bisphosphate as a substrate necessitates the presence of adolase in the assay. This enzyme is present in the 40 – 60% ammonium sulfate fraction of the A protein preparation.

The assay reaction was begun by adding the protein fraction which was to be in excess to the other assay components in order to make a complete mixture for quinolinic acid synthesis *in vitro*. After gentle mixing, tubes were incubated at room temperature (23 °C) without shaking. After 20 min, 0.5 ml of 15% perchloric acid was added to stop the reaction. Then 0.5 ml of 2 mM unlabeled quinolinic acid was added, and the tubes were centrifuged. After decantation, the supernatants were neutralized by addition of 0.5 ml of 2.5 N KOH, with subsequent adjustment to pH 7.0 using indicator paper and were again centrifuged.

The [^{14}C]quinolinic acid in these deproteinized and neutralized reaction mixtures was determined by a minor modification of the method of Chandler and Gholson [8,14].

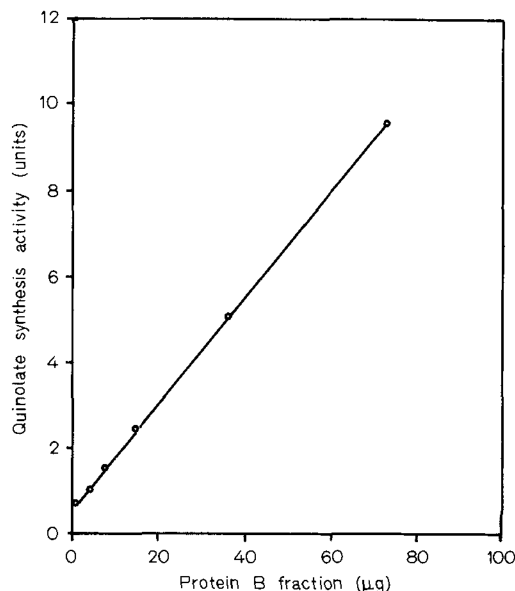

Fig. 2. *The linear dependence of the rate of quinolinate synthesis on the concentration of the B protein.* Aliquots of the 0 – 50% saturation sodium citrate fraction (7.35 mg/ml) were diluted from 1:10 to 1:200 and assayed in duplicate. The measured quinolinate synthetase catalytic activity is plotted against the weight of B protein fraction added

After purification by ion-exchange chromatography, a 2-ml aliquot of the eluate was counted in Bray's scintillation cocktail and the amount of quinolinate synthesized (as nmol) calculated from the specific activity of the L-[U-^{14}C]aspartate added. A unit of enzyme activity is defined as the amount of enzyme which forms 1 nmol of quinolinate from L-aspartate in 20 min at 23 °C under the conditions described.

In order to estimate the amount of B protein present the amount of quinolinate synthesized should be a linear function of the amount of B protein added. This assay satisfied this requirement, as shown in Fig. 2.

Protein Determination

Protein was determined either by the method of Lowry *et al.* [15] or spectrophotometrically by the 280/260-nm absorbance ratio [16].

Chemicals

The L-[U-^{14}C]aspartate (160 mCi/mmol) was obtained from New England Nuclear and from ICN-Isotope and Nuclear Division. All other chemicals were of reagent grade from local supply houses. NAD-agarose was obtained from P-L Biochemicals, Inc.

RESULTS

Purification of the B Protein

Step I. Protamine Sulfate Fractionation. Removal of nucleic acids was accomplished by addition of protamine sulfate to the crude extract. A 2% (20 mg/ml) suspension was prepared by adding 550 mg protamine sulfate to 27.4 ml 0.05 M potassium phosphate. 2 mg protamine sulfate was used to every 10 mg crude extract protein. The suspension was added dropwise with mixing to 415 ml of crude extract kept at 0–4 °C. After the addition was complete and the mixture had been stirred another 30 min, it was centrifuged for 15 min at 8000 × *g*. The pellet was discarded and 443 ml of supernatant were ready for further purification. At all purification steps, 0.5-ml or 1-ml samples were removed and stored at −15 °C for assay and protein determination. Frequently, the total activity recovered in the protamine sulfate supernatant was greater than the assayed activity in the crude extract. This activity enhancement may be due to the removal of inhibiting substances by precipitation with the protamine sulfate. Routinely, this step resulted in recovery of total activity over 90% and a 1–3-fold increase in specific activity over that of the crude extract.

Step II. Sodium Citrate Fractionation. A saturated solution of sodium citrate was prepared at room temperature (23 °C) and adjusted to pH 7.0 with saturated citric acid. Then an equal volume (443 ml) of this solution was added dropwise to the 443 ml of protamine sulfate supernatant which was stirring at 0–4 °C. After completion of this addition and further mixing for 20 min, the suspension was centrifuged 15 min at 8000 × *g*. The supernatant was discarded and the protein pellet was redissolved in 11–12 ml 0.005 M potassium phosphate, pH 7.5, with a final volume of 23 ml. (Sodium citrate was found to inhibit the quinolinate synthesis assay and was removed by dialysis against 0.005 M potassium phosphate pH 7.5 before assay.)

Step III. Gel Filtration on Sephadex G-200. A 4.5 × 88-cm column packed at 4 °C with 1400 ml of swelled, degassed Sephadex G-200 was equilibrated with 2 l of 0.005 M potassium phosphate, pH 7.5. Then 22 ml of the 0–50% saturation sodium citrate fraction was mixed with 1 ml of saturated sucrose solution and layered under the buffer on top of the gel bed. Elution was effected with 0.005 M potassium phosphate buffer, pH 7.5, using a 25-cm head and collecting 9-ml fractions at 4 °C. Flow rate of the column was about 37 ml/h and the column was eluted within 40 h. Previous determinations of the behaviour of the B protein on Sephadex G-200 allowed pooling of fractions containing the B protein without first assaying the effluent. Fractions 68 to 140 were thus pooled, making the pooled eluate 650 ml in volume.

Step IV. Anion-Exchange Chromatography on DEAE-Sephadex. A 4 × 13-cm column of DEAE-Sephadex was packed at 4 °C and equilibrated with 700 ml of 0.005 M potassium phosphate, pH 7.5, and then 647 ml of the pooled Sephadex G-200 effluent was passed through the column. Monitoring the absorbance of the effluent at 280 nm indicated that all of the protein was bound to the DEAE-Sephadex. The column was washed at 4 °C with 0.20 M KCl in 0.005 M potassium phosphate, pH 7.5. After 600 ml of elution, the protein had dropped to below 0.05 absorbance unit at 280 nm in the effluent. This wash was discarded and the column was eluted with 375 ml of 0.25 M KCl, 0.005 M potassium phosphate buffer, pH 7.5, after which the protein absorbance was again reduced to 0.05 absorbance unit. This effluent was a fairly dilute protein solution, so 25-ml and 50-ml portions of glycerol, a stabilizing agent, were added as the 375-ml effluent was collected. The solution was continuously stirred at 4 °C during collection and the final volume was 504 ml, containing 25% glycerol.

Step V. Hydroxyapatite Chromatography. Further purification beyond the DEAE-Sephadex step involved low-protein concentrations and required the presence of 25% glycerol in all buffers to stabilize the B protein activity. A 1.9 × 30-cm column was packed with hydroxyapatite and equilibrated first with 500 ml of 0.005 M potassium phosphate, pH 7.5, and then 200 ml of 0.01 M potassium phosphate, pH 7.5. Both equilibrating solutions contained 25% glycerol. Then 500 ml of the 0.25 M KCl DEAE-Sephadex effluent were passed through the column, and all the protein was bound to the hydroxyapatite. The B protein activity was subsequently eluted with 0.01 M potassium phosphate, pH 7.5, in 25% glycerol, collecting 9.8-ml fractions. The protein peak as monitored by absorbance at 280 nm was pooled and resulted in 78 ml of protein solution. The low flow rate of 25% glycerol solution necessitated running this column at room temperature.

The above purification scheme resulted in an over 340-fold enrichment of specific activity with approximately a quarter of the enzyme activity being recovered from the crude extract, as summarized in Table 2.

Stabilization of the Quinolinate Synthetase A and B Proteins by Glycerol

During the course of the purification, it became apparent that the B protein catalytic activity was unstable in dilute protein solutions. Addition of

Table 2. *Purification of the B protein of the quinolinate synthetase system in* E. coli

Step	Total volume	Total protein	Total activity	Specific activity	Enrichment	Recovery
	ml	mg	units	k-units/mg	-fold	%
Crude extract	416	111659	12242	1.05	1	100
Protamine sulfate supernatant	443	5039	15514	3.08	2.9	127
0–50% sodium citrate pellet	23	837.2	10803	12.9	12.3	88
Sephadex G-200 (pooled fractions)	650	335.0	15470	47.6	45.3	126
DEAE-Sephadex (pooled fractions)	502	55.2	10542	191	182	86
Hydroxyapatite (pooled fractions)	156	8.3	2900	361	344	24

Table 3. *Purification of the A and B proteins from* E. coli nadC *mutant, E-126*
The purifications of the A and B proteins were conducted as described in Materials and Methods. *E. coli* strain E-126 is a *nadC* mutant which is wild-type at both the *nadA* and *nadB* loci

Step	Total volume	Total protein	Total activity	Specific activity	Enrichment	Recovery
	ml	mg	units	k-units/mg	-fold	%
B protein						
Crude extract	194	4656	16199	3.5	1	100
Protamine sulfate supernatant	212	3286	14522	4.4	1.3	89.6
0–50% citrate pellet	7.1	234	5495	23.5	6.7	33.9
DEAE-Cellulose (pooled fractions)	173	52	3689	71.0	20.4	22.8
A protein						
Crude extract	95	2260	1000	0.44	1	100
Heat treatment supernatant	93	2840	804	0.28	0.6	80.4
40–60% ammonium sulfate	18.4	258	405	1.57	3.6	40.5

glycerol greatly retarded the loss of activity at −15 °C, 4 °C and 23 °C and hence it was used in later purification steps. Addition of glycerol to form 33% glycerol solutions of the A protein also stabilized the catalytic activity of the A protein for periods up to 10 days at −15 °C.

Separation of Quinolinate Synthetase into A and B Protein Components

The complementary nature of *nadA* and *nadB* mutant extracts in quinolinate synthesis suggests that two independent enzymes may be required for the net reaction to occur [12]. On the other hand, the physical-chemical characteristics of the mutant enzyme(s) may be dramatically different from the wild-type enzyme. It is conceivable that the mutant recovery procedure used during the mutagenesis experiments could select for complementary enzyme forms and hence introduce natural bias into the character of our mutants. The failure to detect any intermediates between L-aspartate and quinolinate suggests that a complex may be formed between the A protein and the B protein. In order to gain some information about the molecular nature of the native quinolinate synthetase system, the separation of the A protein from the B protein was investigated, starting with a *nadC* mutant which contains both activities. The results of this experiment are presented in Table 3. The salt fractionation employed to partially purify the B protein separates it from the A protein. The heat treatment of 55 °C used to partially purify the A protein inactivates the B protein. The results indicate that the two activities are readily separated from one another. These results suggest the nature of the A · B "complex" is such that the "complexed" forms are in rapid equilibrium with the free forms of the enzyme(s) and make it highly unlikely that the A and B proteins are covalently linked or otherwise tightly bound to one another in this *nadC* mutant which is wild-type at both the *nadA* and *nadB* loci.

Quantitative Estimates of Quinolinate Synthetase A and B Protein Activities in nadA, nadB *and* nadC *Mutants*

The original biochemical classification of quinolinate synthetase mutants into three distinct groups, two mutually complementing groups and one non-complementing group was based upon qualitative

Table 4. *The specific activities of the quinolinate synthetase A and B proteins in* nadA, nadB, nadR *and* nadC *mutants*
The specific activity of the freshly prepared crude extracts was measured as described in Materials and Methods. The percentage activity is compared to the two standard reference mutants, PA-2-18 and SB-16

Mutant	Specific activity		Activity *cf.* PA-2-18 and SB-16	
	A protein	B protein	A protein	B protein
	k-units/mg		%	
nadA				
PA-2-18	48.2	964	5.0	100
AT-11-23	29.6	952	2.3	73.3
nadB				
SB-16	1200	31.7	100	2.7
W-3899	718	31.8	53.4	2.4
nadR				
E-18	86	40		
nadC				
E-126	1150	566	101.5	50.3
SB-99	600	382	51.9	33.2
W-4516	920	672	67.6	49.7

Table 5. *The effect of pyridine nucleotides on quinolinate synthetase activity*
The pyridine nucleotides at the stated concentrations, were added to the standard assay described in the text. The B protein used was purified approximately 150-fold over the crude extract. Results are expressed as units/ml B protein fraction with percentage inhibition (*cf.* control) in brackets

Addition to assay	Quinolinate synthetase activity (inhibition) at pyridine nucleotide concn:		
	3.33 mM	1.0 mM	0.33 mM
	units/ml (%)		
H_2O	513 (0)	513 (0)	513 (0)
NAD^+	22.6 (95.6)	237 (53.9)	437 (10.9)
NADH	41.3 (92.0)	217 (57.8)	414 (19.2)
$NADP^+$	368 (28.3)	366 (28.6)	436 (15.0)
NADPH	393 (23.5)	408 (20.5)	487 (5.0)

enzyme activity measurements conducted with crude extracts [12]. Later, it became apparent that one of these groups of mutants correspond to the *nadA* genetic group. The other complementary group and the non-complementary group are closely linked at the *nadB* and/or *nadR* loci [12]. The quinolinate synthetase assay described in this paper is approximately 10 times more sensitive than the former method using crude extracts. Thus, it was possible to re-examine the original classification schemes using partially purified B to assay for the A protein and *vice versa*. The results, which are shown in Table 4, confirm the general character of the original classification scheme. However, in contrast to earlier results in crude extracts, strain W-3899, which was classified as a *nadR* mutant, is now shown to contain the A protein and is reclassified as a *nadB* mutant. The source of the experimental variation may be due to either the more sensitive assay employed or to further mutations in the strain in the six intervening years.

Effect of the Pyridine Nucleotides on Quinolinate Synthesis

The quinolinate synthetase system may be subject to both repression and feed-back inhibition [6,7]. In order to test this latter hypothesis the effect of the pyridine nucleotides on the partially purified system was investigated. Estimates of the intracellular concentration of NAD in *E. coli* range from 0.3 mM to about 1 mM so this concentration range was studied [17–19]. The results, which are presented in Table 5, indicate that concentrations of NAD which approximate the intracellular concentrations have a dramatic effect on the catalytic activity of quinolinate synthetase. Thus, this experimental evidence supports the hypothesis that this pathway is regulated by feed-back inhibition. In direct contrast to the results obtained with NAD, the experimental data suggest that NADP is not an effector in this system since only weak inhibition is obtained even at concentrations of NADP 10 times above the normal intracellular concentration. Consistent with the NAD inhibition data, we have observed the binding of the B protein to an NAD-agarose affinity column (G. R. Griffith and R. K. Gholson, unpublished observations).

DISCUSSION

In order to study the molecular mechanisms of NAD biosynthesis and its regulation, the individual components of the pathway must be isolated and characterized.

The published experimental data do not provide a basis for determining the character of the enzymic system which synthesizes quinolinate from L-aspartate and dihydroxyacetone phosphate, although the overall stoichiometry of the reaction has been established [7]. In this paper, we present evidence that two proteins which are easily separated are required for quinolinate synthesis. One of these proteins has been purified over 340-fold by salt fractionation, gel filtration and ion-exchange chromatography. The

existence of two protein fractions is consistent with the genetic observations of the *nadA* and *nadB* genes. All our *nadA* mutants contain an enzymically functional B protein. Mutants which map in the *nadB* region, however, may or may not contain the A protein and have been classified as either *nadB* or *nadR* [12]. The nature of *nadR* remains to be established. The failure to detect any significant quinolinate synthetase A or B protein activity in the *nadR* strain can be equally explained by two independent hypotheses. The first hypothesis is that a very tight, but inactive molecular complex is formed between the *nadA* and *nadB* gene products in the *nadR* mutant. In this case, the *nadR* mutation would reside within the structural portion of the *nadB* gene. The second hypothesis is that the *nadR* gene is a true regulator gene which regulates the expression of the non-contiguous *nadA* and *nadB* structural genes. The available biochemical evidence can not distinguish between these two postulates. The published genetic evidence has been interpreted in terms of the true regulatory gene hypothesis. Recently, Tritz [20] reported the isolation of a strain designated as *nadR*$^+$/*nadR* *trans*heterogenate and deduced from regulator gene theory that the *nadR*$^+$ gene is a positive regulator gene since it was *trans* dominant. The genetic evidence was not supported by chemical estimations of the enzyme activities involved. We now show that strain W-3899, (Tritz's strain UTH 4464) which was used to generate the putative *nadR*$^+$/*nadR* *trans*heterogenote is, indeed, a *nadB* mutant (Table 3). Obviously, *nadB*$^+$/*nadB* would be *trans* dominant and the conclusions drawn by Tritz need to be re-evaluated. The possibility that W-3899 has undergone further spontaneous mutations (*i.e.* *nadR* $\rightarrow$ *nadB* in our laboratory or *nadB* $\rightarrow$ *nadR* in Dr Tritz's laboratory) must not be overlooked.

The inhibition of quinolinate synthetase at concentrations of NAD which approximate the normal physiological concentrations of NAD provides further support for the hypothesis that NAD is a feedback inhibitor for the pathway [6]. The observed binding of B protein (but not the A protein) of quinolinate synthetase to an NAD-agarose affinity column may suggest that the molecular site of NAD inhibition of the over-all reaction exists on the B protein of the quinolinate synthetase complex.

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