Nicotinamide Adenine Dinucleotide Glycohydrolases from Ehrlich Ascites Tumor Cell Nuclei: Isolation, Partial Purification, and Properties†

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ABSTRACT: Two forms of nicotinamide adenine dinucleotide glycohydrolase (EC 3.2.2.5) (NADase) have been identified and isolated from Ehrlich ascites tumor cell nuclei. The first, recovered as a DNA complex when the nucleoprotein of sonicated nuclei is precipitated by dilution, is completely inactivated by treatment with DNase, and has a pH optimum of 6.6–7.1. Other properties of this enzyme are: a 50% inhibition in activity by 3.3×10^{-4} M nicotinamide; a 100% inactivation after heating at 45° for 2 min; a 65% loss in activity in the presence of 0.5 M NaCl or more; the inability to hydrolyze $NADP^+$; catalyzes the synthesis of poly(ADPR) from NAD^+. The second nuclear NADase, recovered as a sediment after 1-hr centrifugation at 105,000*g* of a 2 M NaCl solution of sonicated nuclei, is related to the nuclear membrane as shown by sucrose gradient density centrifugation and electron microscopy. Other properties of this enzyme are: a pH–activity maximum between 6.3 and 7.0; stability after heating at 45° for up to 5 min; insensitivity to 1 M NaCl or to treatment with DNase; the absence of the ability to catalyze the synthesis of poly(ADPR) from NAD^+; the ability to hydrolyze $NADP^+$; a 50% inhibition by 9.3×10^{-4} M nicotinamide. This latter finding is in contrast to the 24.3×10^{-4} M nicotinamide which is required to produce the same degree of inhibition in the microsomal enzyme. The possibility that the properties of the membrane-bound nuclear and microsomal NADases may depend on character of the membranes to which they are bound is being investigated.

NAD-glycohydrolase (EC 3.2.2.5) (NADase)[1] hydrolyzes NAD^+ at the ribose–nicotinamide bond (Handler and Klein, 1942) and is found distributed between the microsomes (90–95%) (Jacobson and Kaplan, 1957) and the nuclei (5–10%) (Nakazawa *et al.*, 1968) of the rat liver cell. This enzyme from the nucleus of mammalian cells has been variously described as being similar to (Nakazawa *et al.*, 1968), different from (Yamada *et al.*, 1971), and identical with (Roemer *et al.*, 1968) another nuclear enzyme, namely adenosine diphosphoribose polymerase (ADPR polymerase) (Chambon *et al.*, 1963, 1966). ADPR polymerase, a chromatin-bound enzyme (Yamada *et al.*, 1971; Ueda *et al.*, 1968), requires Mg^{2+} for the successive transfer of the ADPR moieties of NAD^+ with the formation and elongation of a homopolymer composed of several ADPR units (Yamada *et al.*, 1971). Microsomal NADase does not catalyze the polymerization of ADPR moieties of NAD^+ and is different from the nuclear ADPR polymerase insofar as its K_m (NAD^+), pH–activity profile, heat lability and substrate specificity are concerned. These findings have led to the conclusion that microsomal NADase and nuclear NADase are essentially different enzymes (Roemer *et al.*, 1968).

In 1971, Green and Dobrjansky demonstrated that both microsomal and nuclear NADase from Ehrlich ascites cells were irreversibly inactivated by NAD^+ during incubation at 37° for 10 min at pH 8.0. Since the pH optimum for the ADPR polymerase is reported to be 8.0 (Roemer *et al.*, 1968), our findings suggested that ADPR polymerase and nuclear NADase might be two different enzymes. The present experiments on the isolation and characterization of NADase from nuclei of Ehrlich ascites cells have been undertaken in order to compare the properties of this nuclear enzyme with the well-documented properties of microsomal NADase from Ehrlich ascites cells and to gain some understanding of the role that NADase plays in the metabolism of the cancer cell nucleus.

Materials and Methods

Enzyme substrates and reagents were obtained from the following sources: NAD^+, $NADP^+$, nicotinamide, ATP, 2′-deoxyadenylic acid, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase from Sigma Chemical Co., St. Louis, Mo.; twice-crystallized alcohol dehydrogenase (yeast), once-crystallized pancreatic deoxyribonuclease, calf thymus, DNA, Worthington Biochemical Corp., Freehold, N. J.

Ehrlich ascites tumor cells were carried by continuous passage through HA/ICR, 18- to 20-g, male, albino, Swiss mice from which the ascites tumor cells were collected and washed free of erythrocytes (Green and Bodansky, 1962). NADase from the microsomes of these cells was prepared according to the method of Green *et al.* (1969). NADase activity was determined with alcohol dehydrogenase as described in a previous paper (Green and Bodansky, 1965), and the specificity of the enzyme was verified by assay with cyanide (Colowick *et al.*, 1951).

A unit of NADase activity is the amount of enzyme needed to hydrolyze 1 μmole of NAD^+/hr under the specified conditions of the assay. Total protein was determined by the method of Folin as modified by Lowry *et al.* (1951).

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[1] Abbreviations used are: ADPR-polymerase, adenosine diphosphoribose polymerase; NADase, nicotinamide adenine dinucleotide glycohydrolase; G6Pase, glucose 6-phosphatase. All other abbreviations are as listed in *Biochemistry* 5, 1455, 1966.

TABLE I: Fractionation of the NADases of Ehrlich Ascites Cells.[a]

Sample	Total Protein		Total NADase		Total G6Pase		Total DNA	
	Mg	%	Units	%	Units	%	Mg	%
Whole cells	1530	100	280	100	3000	100	39	100
Microsomes	350	23	187	67	2110	70	0	0
Nuclei	130	8	18	6	106	4	33	85
2 M NaCl preparation	17	1	7	3	62	2	0.9	3
DNA preparation	40	3	9	3	0	0	24	62

[a] The microsomal preparation was isolated by centrifugation of the 0.25 M sucrose solution at 105,000*g* after removal of the nuclei and mitochondria. Clean nuclei were isolated by centrifugation through 2.1 M sucrose according to the method of Mamaril *et al.* (1970). See Results for the method of preparation of the 2 M NaCl preparation and the DNA preparation from isolated nuclei.

Results

Isolation of Nuclear NADases. The enzyme source was a nuclear preparation isolated from Ehrlich ascites cells by centrifugation through sucrose density barriers to remove whole cells, microsomes, mitochondria, and debris (Mamaril *et al.*, 1970). The nuclei were judged to be free of contamination by cytoplasmic, mitochondrial, and lysosomal material when appropriate assays showed no measurable activity of those enzymes previously reported to be characteristic of each of these subcellular components (Siebert, 1963). Glucose 6-phosphatase (G6Pase) has been designated as a marker enzyme for microsomes (Siebert and Humphrey, 1965). Since microsomes contain 80–90% of the total cellular NADase activity, the content of G6Pase activity was determined in each nuclear preparation (Swanson, 1955). The presence of nonspecific phosphatases was also determined.

All purification procedures were carried out at 4°. The clean nuclei resulting from the centrifugation through 2.1 M sucrose were washed free of sucrose by twice suspending them in ten volumes of 0.05 M potassium phosphate buffer (pH 7.0), containing 0.01 M EDTA. The nuclei were then suspended in four volumes of the same buffer and disrupted by sonication for 1.0 min in a 100-W, 20-kc M.S.E. ultrasonic disintegrator. Each milliliter of sonicate was diluted with one volume of the phosphate–EDTA buffer and solid NaCl was added with gentle mixing until the final concentration was 2.0 M. During the addition of the NaCl, the precipitate dissolved. After standing for 30 min at 4°, the solution was centrifuged at 105,000*g* for 1 hr and the small gelatinous precipitate was taken up in a volume of the phosphate–EDTA buffer equal to that of the original material. This will be designated as the "2 M NaCl" preparation.

The clear 105,000*g* supernatant solution was brought to 0.15 M NaCl by the dropwise addition of 12.3 volumes of cold, distilled water. After standing for 1 hr, the insoluble nucleoprotein was collected by centrifugation for 30 min at 105,000*g*. The precipitate was suspended by homogenization in a small volume of the phosphate–EDTA buffer (DNA preparation). The results of analysis for total protein, NADase activity, G6Pase activity and DNA (Schneider, 1957) of samples taken during each stage of a typical fractionation procedure are seen in Table I. The amount of hydrolysis of NAD^+ by each fraction as shown in this table was due to NADase, and not pyrophosphatase, and was determined by using the method of Kaplan (1955).

These results demonstrated the existence of two forms of NADase in the nuclei of Ehrlich ascites cells, one associated with the DNA and the other an insoluble form of the enzyme which could be centrifuged out of the 2 M NaCl–buffer mixture at 105,000*g*.

In order to determine whether the material in the 2 M NaCl solution was the result of a partial release of enzyme from the DNA-bound form of the enzyme, a sample of the 2 M NaCl preparation was treated with lipase and the solubilized enzyme was partially purified by $(NH_4)_2SO_4$ precipitation (Green and Bodansky, 1965). This soluble NADase was then mixed with the DNA-bound NADase, and made 2 M with respect to NaCl by the addition of solid salt. After standing for 1 hr at 4°, the mixture was exhaustively dialyzed against isotonic phosphate-buffered saline and the insoluble nucleoprotein was removed by centrifugation. Analysis of the supernatant solution and of a suspension of the DNA-precipitated material showed no loss of soluble NADase. These results indicate that the solubilized NADase did not reassociate with DNA during the precipitation from the isotonic medium. The possibility exists, however, that the solubilization may have rendered the enzyme incapable of reassociation or that all binding sites on the DNA were occupied.

Although the intact nuclei were found free from contamination with other intracellular enzymes, our results did show G6Pase activity in the 2 M NaCl preparation. Siebert and Humphrey (1965) have suggested that the outer nuclear membrane is a continuation of the endoplasmic reticulum. Since the endoplasmic reticulum (*i.e.*, microsomes) is high in G6Pase activity (de Duve *et al.*, 1962), the activity of this enzyme in our nuclei and more specifically in our 2 M NaCl preparation represented either a contamination of the nuclei with microsomal material or a normal component of the cell nucleus.

Characteristics of Nuclear NADases. To establish that the NADases isolated from the nuclei of Ehrlich ascites cells were indeed of nuclear origin, a large preparation of nuclei was made and the two forms of NADase, *i.e.*, the enzyme precipitated from 2 M NaCl by centrifugation (2 M NADase) and the DNA-complexed enzyme (DNA NADase), were isolated. The activity of these enzymes was compared with that of microsomal NADase under a variety of experimental conditions. All values shown were the mean of at least two separate experimental determinations.

pH Optimum. Figure 1 shows the pH–activity profile of the three forms of NADase. As has been previously reported

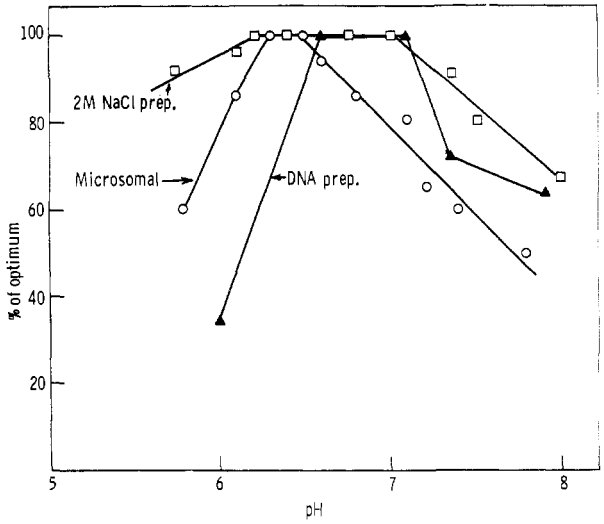


FIGURE 1: The pH–activity profile of the NADase in microsomes and in the 2 M NaCl and DNA preparations. Reaction mixtures contained: 8.0 mM potassium phosphate or glycylglycine buffer; 2.5×10^{-4} M NAD^+ and 2.0 units of NADase[2] in a final volume of 1.0 ml. The value for the pH of each complete reaction mixture was determined with a pH meter before the assay. Microsomal (○); 2 M NaCl preparation (□); DNA preparation (▲).

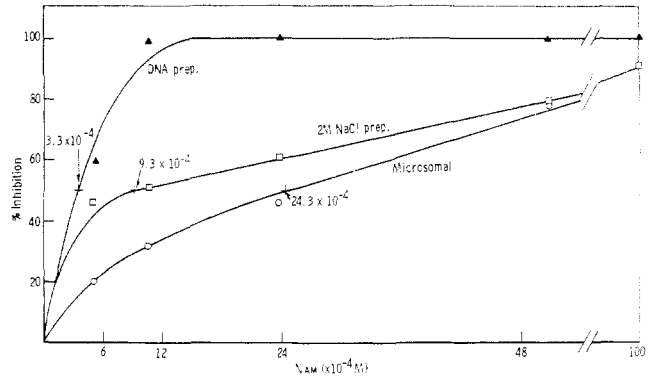


FIGURE 2: Nicotinamide inhibition of NADases. One-milliliter reaction mixtures contained: 8.0 mM potassium phosphate buffer; 2.5×10^{-4} M NAD^+; 2.0 units of each form of NADase; 0, 5.0, 10.0, 25.0, 50.0, or 100×10^{-4} M nicotinamide. The pH of each reaction mixture was 6.5. Microsomal NADase (○); 2 M NaCl preparation (□); DNA preparation (▲).

(Green and Bodansky, 1965), the microsomal enzyme has a sharp optimum between pH 6.3 and 6.5. The pH optima of the NADases in the 2 M NaCl precipitate and the DNA precipitate were rather broad, from 6.3 to 7.0 and 6.6 to 7.1, respectively. The differences between the activities of the enzymes were most apparent below pH 6.5 or above pH 7.0.

Nicotinamide Inhibition. Figure 2 shows the effect of various concentrations of nicotinamide on the activity of 2 M NADase, DNA NADase, and microsomal NADase. Zatman *et al.* (1953), as well as others, have reported that rat liver NADase is inhibited by nicotinamide. Our results show that a 50% inhibition of Ehrlich ascites microsomal NADase occurs in the presence of 24.3×10^{-4} M nicotinamide. In contrast, the concentrations of nicotinamide required to produce a 50% inhibition of NADase in the 2 M NaCl preparation and of the NADase of the DNA preparation were significantly lower and were 9.3×10^{-4} and 3.3×10^{-4} M, respectively.

Heat Lability of NADases. Figure 3 shows the effect of heating the 2 M NaCl preparation, the DNA preparation and the microsomal preparation at 45° for various periods up to 5 min. Whereas the NADase from the 2 M NaCl preparation and from the microsomal preparation were both essentially insensitive to incubation at 45°; in contrast, the NADase in the DNA preparation was extremely labile and was completely inactivated after 2 min.

NADase Activity in the Presence of NaCl. Since the nuclear enzymes were isolated from a 2 M NaCl medium, it was of interest to determine if high salt concentrations influenced the activity of the enzymes. Figure 4 shows that the hydrolysis of NAD^+ by microsomal NADase was essentially unaffected by the presence of NaCl at concentrations up to 1.0 M. The NADase in the 2 M NaCl preparation had 70% of its original activity in 1.0 M NaCl, while the NADase in the DNA preparation had 35% of its original activity in reaction mixtures containing more than 0.5 M NaCl.

[2] All preparations were standardized after assay for NADase by the method described in the text.

Effect of DNase. The loss of activity of the DNA-bound NADase activity from the DNA preparation in 0.5 M or higher NaCl solution suggested that the activity of NADase in this form might require binding to the DNA. Microsomal, 2 M NaCl, and DNA-bound NADases were preincubated with bovine pancreatic DNase for 1 hr in 0.005 M phosphate-buffered saline (pH 7.0), containing 0.01 M $MgCl_2$. Aliquots of each of the reaction mixtures were taken before and after the incubation and were assayed for DNA with diphenylamine according to the method of Schneider (1957). Incubation with DNase did not influence the activity of the microsomal or the 2 M NaCl NADases. In contrast, the NADase in the DNA preparation was completely inactivated. The DNA concentration was found to be less than 10% of its original value.

Substrate Specificity. Equal amounts of microsomal, 2 M NaCl, and DNA NADase activity were incubated with NAD^+ or with $NADP^+$ at pH 6.5 for 4 min at 37°. NAD^+ was determined with alcohol dehydrogenase and ethanol at pH 9.0 and $NADP^+$ was determined with glucose-6-phosphate dehydrogenase and glucose 6-phosphate, at pH 7.4. The hydrolysis of $NADP^+$ by each preparation of NADase, relative to the hydrolysis of NAD^+, was: for microsomal NADase, 80%; for 2 M NaCl NADase, 59%; and for DNA-bound NADase, 0%.

ADPR Polymerase Activity. Aliquots of microsomal, 2 M NaCl, and DNA preparations which assayed 3.0 units of NADase activity/ml at pH 6.5 were assayed for ADPR polymerase activity. For this, each of the enzyme preparations in Tris buffer (pH 7.4) was incubated for 15 min at 25° with optimal concentrations of DNA, histones, Mg^{2+}, and [^{3}H]NAD. The reaction was stopped by adding cold trichloroacetic acid and the incorporation of the [^{3}H]adenine moiety of [^{3}H]NAD into the trichloroacetic acid precipitated material was measured (Burzio and Koide, 1971). The results showed no radioactivity was incorporated into this acid-insoluble material from reaction mixtures with either the microsomal NADase preparations. In contrast, significant incorporation of radioactivity was found in the acid-insoluble precipitate from the reaction mixture containing the DNA-bound NADase. The incorporation of radioactivity into the acid-insoluble material was Mg^{2+} dependent and was proportional to the amount of enzyme used.

Characterization of the Material in the 2 M NaCl Preparation. Franke *et al.* (1970) recently published a report on the

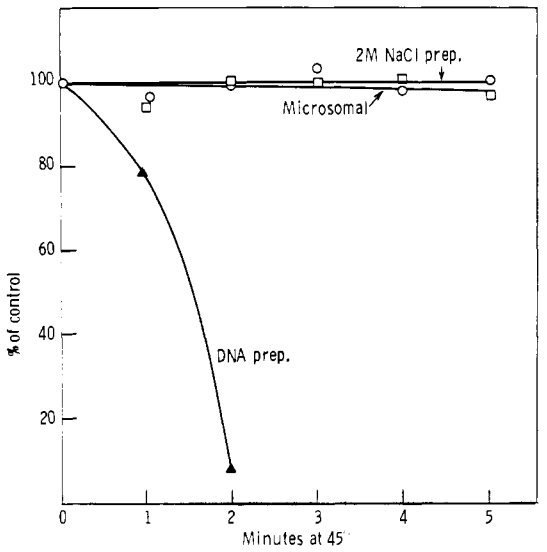


FIGURE 3: Heat lability of NADases. Suspensions of each of the preparations containing 2.0 units of NADase/ml were held at 45° for the times shown. The residual NADase activity was then determined by the standard assay procedure. Microsomal NADase (○); 2 M NaCl preparation (□); DNA preparation (▲).

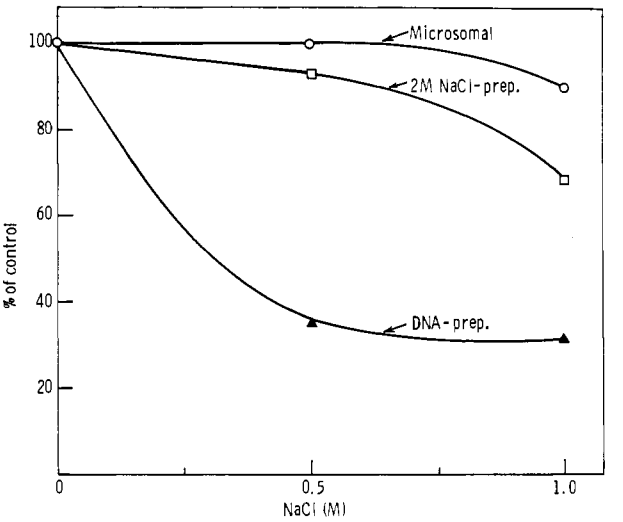


FIGURE 4: Effect of NaCl on the activity of NADases. In addition to the components of the reaction mixtures at pH 6.5, each contained from 0 to 1.0 M NaCl. Incubation of the NADases with NAD^+ in the presence of one of these salt concentrations was for 4 min at 37°. NAD^+ was determined with alcohol dehydrogenase as described in Methods. Microsomal (○); 2 M NaCl preparation (□); and DNA preparation (▲).

isolation procedure and general characterizations of nuclear membranes from mammalian liver which involved the use of hypertonic salt. Since the presence of G6Pase has been considered to represent the presence of membranous material of the cell, it was of interest to determine if the NADase in the 2 M NaCl fraction (see Table I) which we separated from the Ehrlich ascites cell nucleus was associated with the nuclear membrane. For comparison, the plasma membrane and the microsomal membrane (endoplasmic reticulum) from the Ehrlich ascites tumor cells were also isolated.

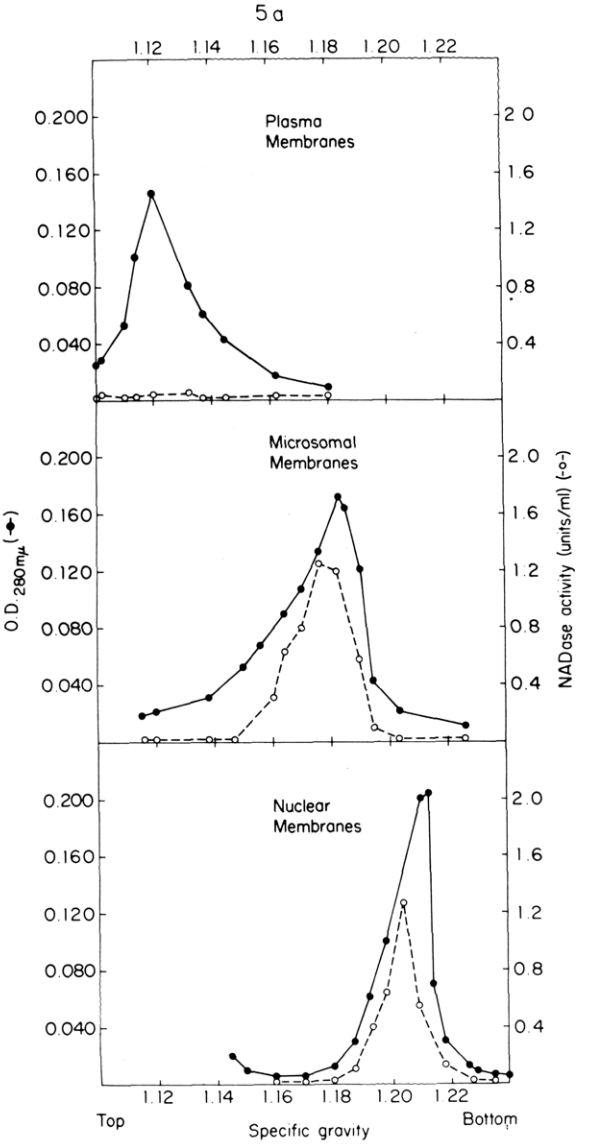
Plasma Membranes. Ehrlich ascites cells were allowed to swell in ten volumes of 2 mM EDTA (pH 7.0) for 10 min and were disrupted by ten strokes of a tight-fitting pestle in a Dounce homogenizer. The homogenate was added to an equal volume of buffered 0.25 M sucrose and was centrifuged for 15 min at 2000 rpm. The pellet was suspended by homogenization in 10 ml of buffered 1.7 M sucrose solution and layered over an equal volume of 2.1 M sucrose. Eight milliliters of 0.85 M sucrose was then layered over the 1.7 M sucrose, and the tube was centrifuged in an SW 25.1 rotor at 24,000 rpm for 30 min. This procedure resulted in the packing of the plasma membranes at the 0.85–1.7 M sucrose interface. Debris, intact cells, and broken nuclei collected at the 1.7–2.1 M sucrose interface and a clean gelatinous pellet of nuclei collected at the bottom of the 2.1 M sucrose layer. The material at the 1.7–2.1 M sucrose interface was collected, suspended by homogenization in 0.85 M sucrose, and layered over 1.7 M sucrose. After centrifugation at 24,000 rpm, for 30 min in an SW 25.1 rotor, the material at the 0.85–1.7 M sucrose interface was collected and pooled with that collected in the initial centrifugation.

The total preparation from the 0.85 M sucrose interface was suspended in 0.25 M buffered sucrose and centrifuged for 30 min at 2500 rpm. The precipitate was washed several times by suspension in 0.01 M potassium phosphate buffer (pH 7.4) containing 0.07 M KCl and was finally sonicated for 1 min in a small volume of the same buffer. The suspension was made 2 M with respect to NaCl and after standing at 4° for 30 min was centrifuged for 1 hr at 105,000*g*. The pelleted membranes (PM) were suspended in phosphate buffered KCl.

Microsomal Membranes. The supernatant solution which resulted from centrifugation of the homogenate at 2000 rpm was made 0.25 M with respect to sucrose and was centrifuged for 15 min at 8000*g* to remove mitochondria. The microsomes in the 8000*g* supernatant solution were collected by centrifugation at 105,000*g* for 2 hr, taken up in phosphate-buffered KCl, and sonicated. NaCl was added to the sonicated microsomal suspension to a final concentration of 2 M. After standing at 4° for 30 min, the suspension was centrifuged for 60 min at 105,000*g* and the pelleted microsomal membranes (MM) were taken up in phosphate-buffered KCl.

Aliquots of the 2 M NaCl preparation, PM preparation and MM preparation were suspended in a 20% (w/w) sucrose solution made up in 0.07 M KCl–0.01 M potassium phosphate buffer (pH 7.4) placed on a 20–55% continuous sucrose gradient (w/w) and centrifuged for 4.5 hr at 80,000*g* in an SW 39 rotor. Twenty drop fractions were collected after puncturing the bottom of each tube with a short 22-gauge needle. The protein, NADase and specific gravity of the material in each sample were then determined.

Figure 5a shows the density distribution of typical nuclear, microsomal and plasma preparations on a 20–55% (w/w) sucrose gradient. The plasma membrane preparation had a peak density, ρ, of 1.121 and the material present in this fraction had no measurable NADase activity. In contrast, both the microsomal and nuclear membrane fractions which had densities of 1.184 and 1.213, respectively, contained NADase activity. The NAD^+ pyrophosphatase activity was also measured (Skidmore and Trams, 1970). The relative total NADase and NAD^+ pyrophosphatase activities in each sample were 0 and 100% for the plasma membrane, 75 and 25% for the microsomal membrane, and 85 and 15% for the nuclear membrane preparations. For visual comparison, the 2 M NaCl, MM, and PM preparations were examined in an electron microscope. Samples of the 2 M NaCl preparation (nuclear), the MM preparation (microsomal) and the PM



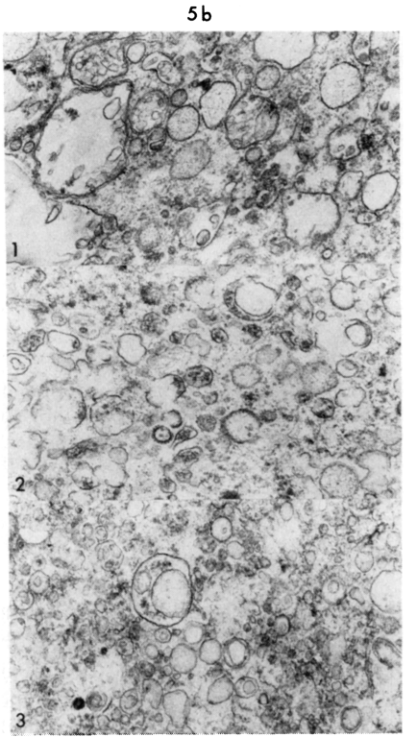


FIGURE 5: Density distribution (a) and electron photomicrographs (b) of plasma, microsomal and nuclear membranes from Ehrlich ascites cells. (a) The sucrose gradient (●) was 20–55% (w/w) in 0.01 M potassium phosphate buffer (pH 7.4) and 0.07 M KCl. Each preparation was suspended in 20% sucrose and was layered on top of the preformed gradient. Centrifugation was for 4.5 hr at 28,000 rpm in an SW 39 rotor. Samples were prepared and analyzed as has been described in the text. NADase activity (○) of the samples was determined with alcohol dehydrogenase (see Methods). (b) Electron photomicrographs of material in each preparation from Ehrlich ascites tumor cells: (1) plasma membranes (PM preparation) × 58,000; (2) microsomal membranes (MM preparation) × 58,000; (3) nuclear membranes (2 M NaCl preparation) × 58,000. See text for method of fixing and staining.

preparation (plasma) were pelleted by centrifugation at 2500 rpm for 30 min and fixed in a mixture of acrolein and glutaraldehyde for 1 hr. The pellets were rinsed with phosphate buffer, diced, postfixed in *s*-collidine-buffered 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. Thin sections which were stained with uranyl acetate followed by lead citrate were examined in a Siemans electron microscope. In Figure 5b are shown electron photomicrographs of (1) the plasma membrane (PM), (2) microsomal membrane (MM), and (3) nuclear membrane (2 M NaCl) preparations, prepared as described in the text for use in the determination of the specific gravity of membranes by sucrose density gradient centrifugation (see Figure 5a). The size and shape of the smooth vesicles are typical of membrane structures and are comparable to those observed by others (Franke *et al.*, 1970; Wood *et al.*, 1970; Wallach and Kamat, 1964).

Discussion

We have recently reported that NADases from tissues of several mammalian species have different properties and may thus be divided into two classes (Green and Dobrjansky, 1971). Preliminary investigations have indicated that differences also exist between the NADases of subcellular organelles (Mamaril *et al.*, 1970). This paper deals with the isolation and characterization of two NADases from Ehrlich ascites cell nuclei and a comparison of the properties of these to the enzyme from microsomes.

Two distinct forms of NADase have been demonstrated in nuclei of Ehrlich ascites tumor cells. The first, a DNA-bound NADase, is heat labile, is rapidly and irreversibly inactivated by high NaCl concentrations or by treatment with DNase, is inhibited to the extent of 50% by 3.3×10^{-4} M

nicotinamide, and has a pH optimum of 6.6 to 7.1. This preparation also contains ADPR polymerase activity. Recently, Miwa and Sugimura (1971) reported the presence of a DNA-bound glycohydrolase in the nuclei from calf thymus which degrades poly(ADPR) by hydrolysis at the ribose–ribose linkage. The possibility exists that this glycohydrolase and the DNA-bound NADase from Ehrlich ascites cells may be related.

The second nuclear NADase is bound to the nuclear membrane (2 M NaCl NADase) and may of its properties strongly resemble those of the corresponding microsomal enzyme. This nuclear enzyme is heat stable, is insensitive to high NaCl concentrations, is not inactivated by treatment with DNase, has a pH optimum between 6.3 and 7.0, and does not catalyze the synthesis of poly(ADPR) from NAD^+. The only significant difference between the nuclear and microsomal membrane bound NADases appears to be the degree of their sensitivity to inhibition by nicotinamide.

The buoyant densities of nuclear and microsomal membranes from rat liver and from Ehrlich ascites cells as determined by sucrose density centrifugation have been reported to range between 1.16 and 1.20 (Zbarsky *et al.*, 1969; Franke *et al.*, 1970). Plasma membranes from Ehrlich ascites cells have a buoyant density of about 1.10 (Kamat and Wallach, 1965). These results are in agreement with our present findings. That NADase activity is present at 1.18 in the microsomal fraction and 1.21 in the nuclear fraction (Figure 5a) confirms the fact that one form of NADase is bound to membranes in these subcellular structures of Ehrlich ascites tumor cells. We did not find NADase in the plasma membrane of the Ehrlich ascites cell, a result which is at variance with the results of Bock *et al.* (1971) on rat liver plasma membranes. This may be due to the differences between the plasma membrane of this tissue and of the Ehrlich ascites cell. Our finding that the plasma membranes of Ehrlich ascites cells had considerable NAD^+ pyrophosphatase is in accord with the findings of Emmelot *et al.* (1964) and of Skidmore and Trams (1970).

Since the membranes of the nuclei and the microsomes of Ehrlich ascites cells have different physical and biochemical properties (see Figure 5a; Franke *et al.*, 1970), the possibility exists that the particular nature of the enzyme–membrane complex may determine some of the properties of the enzyme. This possibility is currently under investigation.

Acknowledgments

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