NICOTINAMIDE ADENINE DINUCLEOTIDE GLYCOHYDROLASES AND POLY ADENOSINE DIPHOSPHATE RIBOSE SYNTHESIS IN RAT LIVER*

Kinya Nakazawa**, Kunihiro Ueda, Tasuku Honjo,
Koichiro Yoshihara+, Yasutomi Nishizuka and Osamu Hayaishi

Department of Medical Chemistry
Kyoto University Faculty of Medicine
Kyoto, Japan

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Mammalian NAD glycohydrolase (NADase) has been established to catalyze two different reactions: (a) hydrolysis of NAD to ADP-ribose and nicotinamide, and (b) transfer of the ADP-ribose moiety of NAD to some pyridine derivatives such as acetylpyridine, thionicotinamide, and nicotinamide (exchange reaction) (Zatman et al., 1953). In a preceding paper (Nishizuka et al., 1968), a chromatin fraction obtained from rat liver has been shown to transfer the ADP-ribose moiety of NAD to histone. A successive transfer of this moiety results in the formation and elongation of a homopolymer composed of several ADP-ribose units (poly ADP-ribose synthesis) (Chambon et al., 1966; Nishizuka et al., 1967; Sugimura et al., 1967). The enzyme responsible for this reaction, which will be

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** Present address: Department of Psychiatry, Kyoto University Faculty of Medicine, Kyoto, Japan.

+ On leave from Institute of Cancer Research, Kyushu University School of Medicine.

tentatively referred to in this paper as ADP-ribose transferase, may be related to an NADase since nicotinamide is released during the reaction. The present studies were undertaken to identify different NADases in rat liver, and to further explore the mechanism of poly ADP-ribose synthesis.

MATERIALS AND METHODS

Rat liver nuclei were isolated by the method of Chauveau et al. (1956), and further washed with 2.2 M sucrose containing 3.3 mM $CaCl_2$. Chromatin was prepared from the nuclei as described previously (Ueda et al., 1968). Rat liver microsomes were prepared by the method of Schneider and Hogeboom (1950). Endoplasmic membrane was isolated from the microsomes by the method of Chauveau et al. (1962). Various radioactive NAD's were prepared as described previously (Nishizuka et al., 1967).

ADP-ribose transferase was assayed by measuring the acid-insoluble radioactivity with NAD-(adenine-8)-^{14}C as a substrate as described earlier (Ueda et al., 1968). The reaction mixture contained 16 mμmoles of NAD-^{14}C (2,260 cpm/mμmole), 7.5 μmoles of $MgCl_2$, 25 μmoles of Tris-Cl buffer, pH 8.0, and an enzyme preparation in a total volume of 0.25 ml. Incubation was carried out for 10 min at 37°. The NADase activity was assayed under the identical conditions except that NAD-(nicotinamide-7)-^{14}C (8,500 cpm/mμmole) was employed as a substrate. The radioactive nicotinamide produced was isolated by paper chromatography with 1 M ammonium acetate, pH 5.0-ethanol (3:7) as a solvent, and was determined by direct paper-strip counting with a Packard Tri-Carb liquid scintillation spectrometer.

EXPERIMENTAL RESULTS

In agreement with the earlier observations by Jacobson and Kaplan (1957), a major portion (more than 90%) of the cellular NADase activity was localized in microsomes, and only about 5% was recovered in nuclei. Upon further fractionation of microsomes, most of the activity was found in endoplasmic membrane (microsomal NADase). In contrast, the ADP-ribose transferase activity was exclusively localized in chromatin (Ueda et al., 1968). In addition to the polymer synthesis, the chromatin fraction hydrolyzed NAD simply to ADP-ribose and nicotinamide (chromatin NADase). With chromatin, quantitative analyses showed that approximately 20-30% of nicotinamide released was accounted for by the ADP-ribose incorporated into acid-insoluble material. The addition of either histone, DNA, or both to the microsomal fraction did not induce the polymer synthesis, and mixing the chromatin and microsomal fractions decreased the polymer synthesis by the former fraction alone.

Table I summarizes the properties of microsomal and chromatin NADases as well as ADP-ribose transferase. Both microsomal and chromatin NADases were equally inhibited by various pyridine derivatives, such as nicotinamide, acetylpyridine and thionicotinamide. These compounds also inhibited concomitantly the ADP-ribose transferase activity (Nishizuka et al., 1967; Sugimura et al., 1967). The NADases and ADP-ribose transferase were relatively insensitive to nicotinic acid and isonicotinic acid hydrazide. The inhibition of NADase by a pyridine derivative was shown to be ascribed to an exchange reaction of the nicotinamide moiety of NAD with that compound, resulting in the formation of an NAD analogue (Zatman et al.,

Table I

Comparison of NADases and ADP-ribose Transferase

Properties	Microsomal NADase	Chromatin NADase	ADP-ribose transferase
Inhibitor	Pyridine derivatives	Pyridine derivatives	Pyridine derivatives
Exchange reaction	Positive	Positive	----
Km for NAD	1.7×10^{-4} M	2.5×10^{-4} M	2.5×10^{-4} M
Optimum pH	6.4	7.6 - 8.0	8.0
Heat stability	Stable	Labile	Labile
Specificity	NAD, NADP	NAD	NAD
DNase treatment	Resistant	Sensitive	Sensitive

1953). Indeed, both microsomal and chromatin NADases catalyzed the exchange reaction with radioactive nicotinamide but not with nicotinic acid.

Chromatin NADase and ADP-ribose transferase showed an identical Km value for NAD, which was slightly higher than that of microsomal NADase. The microsomal enzyme showed the maximum activity at pH 6.4, in contrast to chromatin NADase and ADP-ribose transferase which were most active at pH 7.6-8.0[1/]. The enzymes associated with chromatin were rather labile, and about 75% of the activities was lost upon heat treatment at 45° for 1 min at pH 7. In contrast, microsomal NADase was stable under the above condition. In support of the finding that chromatin NADase showed somewhat different properties from microsomal NADase as described above, these two enzymes were

1/ Similar observations on microsomal and nuclear NADases obtained from Ehrlich ascites cells have recently been made by Roemer et al. (1967) (also personal communication from Dr. V. Roemer).

Table II

Substrate Specificities of Microsomal and Chromatin NADases and ADP-ribose Transferase

The activities were assayed under the standard conditions except various substrates were used as indicated. Radioactive NADP was prepared from NAD-^{14}C with pigeon liver NAD kinase (Wang and Kaplan, 1954). Deamido-NAD was prepared as described previously (Nishizuka and Hayaishi, 1963). Acetylpyridine-NAD was prepared by an enzymic exchange reaction with beef spleen NADase (Kaplan, 1955).

Substrate	Microsomal NADase	Chromatin NADase	ADP-ribose transferase
NAD	100	100	100
NADP	51	0	0
Deamido-NAD	0	0	0
Acetylpyridine-NAD	35	4	4

Numbers are expressed as relative activities (NAD:100).

clearly distinguished by substrate specificities. As shown in Table II, the microsomal enzyme split NADP as well as NAD and acetylpyridine-NAD. In contrast, chromatin NADase was essentially inactive for NADP, which was also inert as a substrate for ADP-ribose transferase. The poly ADP-ribose synthesis was previously shown to be inhibited by a prior treatment of chromatin with pancreatic DNase but not with RNase (Chambon et al., 1966; Nishizuka et al., 1967). Similarly, the chromatin NADase activity was found to be apparently sensitive to DNase but not to RNase. The microsomal enzyme, however, completely resisted the DNase treatment under the same condition.

Table III

Effects of DNase and RNase on Enzyme Activities

The enzyme activities were assayed under the standard conditions, except that the enzyme preparations were preincubated with 200 µg of either pancreatic DNase or RNase for 10 min at 37°. The reaction was started by the addition of the substrate.

Treatment	Microsomal NADase		Chromatin NADase		ADP-ribose transferase	
	Activity*	(%)	Activity*	(%)	Activity*	(%)
none	194.6	100	36.9	100	14.8	100
DNase	193.4	100	10.7	29	2.6	18
RNase	194.6	100	36.7	100	14.7	100

* Activity is expressed as mµmoles per mg of microsomal or chromatin protein per 10 min under the standard conditions.

DISCUSSION

The results presented above indicate that at least two different NADases may be distinguished. One enzyme localized in endoplasmic membrane catalyzes the hydrolysis of NAD as well as exchange reaction. The other enzyme associated with chromatin comprises approximately 5% of the total cellular NADase activity, and shows slightly different properties: a higher optimum pH and a higher Km value for NAD. It does not react with NADP and is sensitive to DNase. The enzyme responsible for the poly ADP-ribose synthesis is also localized in chromatin, and seems to belong to the entity designated thus far NADase, since the polymer synthesis is a transglycosidase reaction with the simultaneous release of nicotinamide. Close similarities of the latter enzyme and the NADase associated with chromatin may suggest that chromatin NADase plays an essential role

in the poly ADP-ribose synthesis. More purified enzyme preparations will be used to examine the mechanism as well as the precise role of chromatin NADase in the polymer synthesis.

During the preparation of this manuscript, Dr. K. W. Bock communicated to us that similar results were obtained with nuclear and microsomal NADases obtained from Ehrlich ascites cells (Bock et al., 1968).

REFERENCES

Bock, K.W., Gäng, V., Beer, H.P., Kronau, R., and Grunicke, H., Europ. J. Biochem., in press (1968).

Chambon, R., Weill, J.D., Doly, J., Strosser, M.T., and Mandel, P., Biochem. Biophys. Res. Commun., 25, 638 (1966).

Chauveau, J., Moulé, Y., and Rouiller, C., Exp. Cell Research, 11, 317 (1956).

Chauveau, J., Moulé, Y., Rouiller, C., and Schneebeli, J., J. Cell Biol., 12, 17 (1962).

Jacobson, K.B., and Kaplan, N.O., J. Biophys. Biochem. Cytology, 3, 31 (1957).

Kaplan, N.O., in S.P. Colowick and N.O. Kaplan (Editors), Methods in enzymology, Vol. II, Academic Press, New York, 1955, p. 660.

Nishizuka, Y., and Hayaishi, O., J. Biol. Chem., 238, 3369 (1963).

Nishizuka, Y., Ueda, K., Nakazawa, K., and Hayaishi, O., J. Biol. Chem., 242, 3164 (1967).

Nishizuka, Y., Ueda, K., Honjo, T., and Hayaishi, O., J. Biol. Chem., in press (1968).

Roemer, V., Preiss, J., and Hilz, H., VII Internatl. Congress of Biochem., Tokyo, Abstract Vol. IV, p. 810 (1967).

Schneider, W.C., and Hogeboom, G.M., J. Biol. Chem., 183, 123 (1950).

Sugimura, T., Fujimura, S., Hasegawa, S., and Kawamura, Y., Biochim. Biophys. Acta, 138, 438 (1967).

Ueda, K., Reeder, R.H., Honjo, T., Nishizuka, Y., and Hayaishi, O., Biochem. Biophys. Res. Commun., in press (1968).

Wang, K., and Kaplan, N.O., J. Biol. Chem., 206, 311 (1954).

Zatman, L.J., Kaplan, N.O., and Colowick, S.P., J. Biol. Chem., 200, 197 (1953).