SOLUBILIZATION AND SOME PROPERTIES OF NAD-GLUCOHYDROLASE ISOLATED FROM RABBIT HEART

G. M. Boriskina and L. A. Tseitlin

UDC 612.173.1.015.16:577.154.22

Methods of isolating NAD-glucohydrolase from the microsomal fraction of the heart were tested. NAD-glucohydrolase was found to pass into the soluble state under the influence of phospholipase A, Triton X-100, and Na cholate. NAD-glucohydrolase from heart muscle has unique properties: it is reversibly denatured by 6 M urea but is not inactivated by its own substrate (NAD) at pH 8.0; reversible denaturation by urea correlates for NAD-glucohydrolases isolated from other sources as a rule with inactivation of the enzyme in the presence of NAD at pH 8.0.

KEY WORDS: NAD-glucohydrolase and its properties; microsomes of the heart.

NAD-glucohydrolase, or NADase (E.C. 3.2.2.5), is an enzyme that hydrolyzes NAD at the riboside bond with the formation of free nicotinamide and adenosinediphosphate-ribose. Since the NADases of animal tissue are firmly bound with the membranous structures of the cell, the task of isolating and purifying them requires solubilization of the enzyme [5, 10, 11]. Data in the literature indicate that high concentrations of urea (6 M), as well as NAD, have different effects in an alkaline medium on NADases isolated from different sources [6, 7]. Rabbit heart NADase differs from the NADases of other animal tissues in a number of special properties: it is easily inactivated by photooxidation [2], it has a comparatively low transglucosidase activity [1] and a high molecular weight, and it is not inhibited by adenine derivatives in concentrations of the order of 10^{-3} M.

The object of this investigation was to continue the study of the properties of rabbit heart NADase.

EXPERIMENTAL METHOD

The microsomal fraction of the heart was isolated by a modified Zatman's method [3]. NADase was solubilized by phospholipase A from venom of the cobra Naja naja in 0.05 M phosphate buffer, pH 7.2, at 37°C for 3 h; the ratio between the protein level in phospholipase A and the protein content in the microsomes was 1:40. The microsomal fraction was solubilized with 0.2% Triton X-100 or 1% Na cholate in 0.05 M phosphate buffer, pH 7.2, at 0°C for 40 min. The samples were then centrifuged at 105,000 g for 1 h to yield the corresponding extract and residue. The enzyme activity was determined by enzymic (with alcohol dehydrogenase) and cyanide methods [4]. Protein was determined by Lowry's method [9].

Disc electrophoresis was carried out in 6.5% polyacrylamide gel for 3 h using a current of 2-3 mA to the tube, with tris-glycine buffer, pH 8.3, as the electrode buffer [8]. NADase activity was determined after elution of the enzyme with 0.05 M maleate buffer, pH 6.0. The effect of NAD on the enzyme activity was studied in the microsomal fraction in 0.05 M tris-HCl buffer, pH 8.0 [7].

M. V. Lomonosov Moscow State University. Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, S. E. Severin.) Translated from Byulletein' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 1, pp. 34-36, January, 1975. Original article submitted February 14, 1974.

TABLE 1. Solubilization of Rabbit Heart NADase

Solubilizing agent	Sample tested	Specific activity (μmoles/kg/h)	Total activity (μmoles/h)	Deg. of solubilization of activity (%)	Protein (mg/ml)	Total protein (mg)	Deg. of solubilization of protein (%)	Degree of purification
Triton X-100 (0.2%)	Microsomes	1,96	32,7		2,78	16,7		
	Extract	3,96	26,2	80	1,1	6,6	39	2,0
	Residue	0,50	4,86		1,88	9,65		
Na cholate (2%)	Microsomes	1,6	3,94		2,96	2,46		
	Extract	3,3	3,16	81	1,15	0,96	39	2,0
	Residue	0,43	0,57		2,56	1,28		
Phospholipase A	Microsomes	1,9	6,17		2,73	3,25		
	Extract	2,03	3,68	61	1,56	1,86	57	1,07
	Residue	1,3	2,1		2,52	1,6		

EXPERIMENTAL RESULTS

Solubilization of the NADase isolated from heart muscle was carried out by treating the microsomal fraction with phospholipase A, and also with the detergents Triton X-100 and Na cholate. As Table 1 shows, phospholipase A transformed 60% of the total enzyme activity into the soluble state without causing any change in its specific activity in the supernatant. The detergents solubilized 80% of the activity and doubled the specific activity of the enzyme.

Unlike the enzymes from other animal tissues, NADase isolated from rabbit heart cannot be solubilized by trypsin or lipase, and not by mechanical disintegration of the microsomes (repeated freezing and thawing, treatment with ultrasound). On treatment of the microsomal fraction with trypsin, not NADase but 50-60% of ballast proteins were converted into the soluble state; the specific activity in the residue was doubled. Further treatment of the residue with Triton X-100 led to solubilization of 70% of the total activity, as a result of which the NADase was purified by 34 times compared with the homogenate. However, further purification of the preparations thus obtained was made difficult because of the impossibility of removing all the Triton X-100. Treatment of the residue obtained after the action of trypsin and phospholipase A led to only very slight solubilization of the NADase.

The results of electrophoretic fractionation in 6.5% polyacrylamide gel showed that NADase preparations solubilized by Triton X-100 and phospholipase A possessed identical electrophoretic mobility. These findings suggest that solubilizing agents differing so much in the nature of their action as Triton X-100 and phospholipase convert NADase into the soluble state in the same form.

Investigation of the effect of 6 M urea on the activity of NADase from heart muscle showed that the enzyme is completely inactivated under these conditions. However, the inactivation was reversible: after removal of the urea (by dilution) the activity of the enzyme was almost completely (by 85%) restored. Similar reversible inactivation has been described for NADase from rat liver and attributed to the appearance of different subunit forms of the enzyme [6]. The possibility cannot be ruled out that dissociation–association of the enzyme also occurred under the conditions of the present experiments, more especially since NADase from heart muscle has a molecular weight of the order of 300,000. Experiments to study the effect of NAD at pH 8.0 on the activity of heart muscle NADase showed that the enzyme is not inactivated by its own substrate.

These results show that in several of its properties NADase from heart muscle possesses definite features of similarity with NADases isolated from other sources. Differences in their properties are seen particularly clearly when their molecular weight and their ability to be converted into the soluble state by the action of certain solubilizing agents are compared. This evidently indicates that heart muscle NADase is built into the membranous structures of the cell in a special way, and also that there are certain differences in the molecular organization of the tissue NADases.

LITERATURE CITED

1. G. M. Boriskina and L. A. Tseitlin, Dokl. Akad. Nauk SSSR, 207, No. 2, 464 (1972).
2. A. Z. Prikhod'ko, Byull. Éksperim. Biol. i Med., No. 12, 23 (1973).
3. A. Z. Prikhod'ko, L. A. Tseitlin, and S. E. Severin, Biokhimiya, 39, 123 (1974).

4. S. E. Severin, L. A. Tseitlin, and T. N. Druzhinina, Biokhimiya, 28, 145 (1963).
5. S. G. Alivisatos and D. W. Woolley, J. Biol. Chem., 219, 823 (1956).
6. S. Green, Hoppe-Seylers Z. Physiol. Chem., 353, 851 (1972).
7. S. Green and A. Dobrjansky, Biochemistry, 10, 4533 (1971).
8. B. I. Davis, Ann. New York Acad. Sci., 121, 404 (1964).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. N. O. Kaplan, Biochim. Biophys. Acta, 56, 388 (1962).
11. N. Swislocki and N. O. Kaplan, J. Biol. Chem., 242, 1083 (1967).