Urey, J. C. and D. B. Smith. Effects of some glycosidases and of periodate on the activity of the glycoprotein NAD(P)ase.

Everse et al. (1975 Arch. Biochem. Biophys. 169: 702) extended their earlier report that Neurospora NAD(P)ase (E.C.3.2.2.5) is a glycoprotein containing 80% carbohydrate by weight. Field et al. (1973 Abst. Am. Soc. Microbiol. Mtg.) reported that the enzyme reacts specifically with Concanavalin A demonstrating α-mannose or ~-glucose as a terminal residue in the carbohydrate portion. We report here the effects of several glycosidases and of periodate oxidation upon the enzymic activity of NAD(P)ase.

Strain 74-OR23-1A was grown on solid Vogel's medium N plus 2% sucrose to obtain conidia. Enzyme was obtained by washing the conidia with 0.1 M sodium phosphate buffer pH7.5 and removing the conidia by centrifugotion. Enzyme was also extracted from cultures grown on zinc-deficient Fries minimal medium (Kaplan et al. 1951 J. Biol. Chem. 188: 397). After harvest, the mycelium was homogenized in phosphate buffer pH7.5 and the debris removed by centrifugotion. These different crude enzyme preporations gave indistinguishable results. NAD(P)ase activity was assayed by the cyanide-addition method of Kaplan et al. (1951). Ficin α-galactosidase (generously provided by Dr. Su-chen Li, Tulane University) was incubated with NAD(P)ase at 25° C in 0.5 M sodium acetate buffer pH4.5 for up to 18 hours. Jack Bean α-mannosidase (gift of Dr. Li) was incubated with NAD(P)ase at 25° C in 0.05 M acetate buffer pH 4.5 for up to 34 hours. E. coli β-galactosidase was incubated with NAD(P)ase at 37° C in 0.1 M sodium phosphate buffer pH 7.0 plus 0.1 M mercaptoethanol, 1 mM MgS04 and 0.2 mM $MnSO_4$ for up to 37 hours. Rhizopus sp. α-glucoamylase (Sigma) was incubated with NAD(P)ase at 37° C in 0.1 M acetate buffer pH4.5 plus 1 mM phenylmethyl sulfonyl fluoride (PMSF) for up to 14.5 hours. B. subtilis α-amylase (Sigma) was incubated with NAD(P)ase at 25°C in 0.02 M phosphate buffer pH 6.9 plus 1 mM PMSF for up to 14.5 hours.

In every experiment with each of these five glycosidases, no effect of the glycosidase on the activity of NAD(P)ase was detected. Since the NAD(P)ase was not pure, we were unable to determine whether any sugar residues hod been released from the enzyme. We report in Table I data showing that PMSF does not inhibit NAD(P)ase suggesting that none of the eight serine residues in the enzyme is important for its activity. In the absence of PMSF, the proteinases in the Rhizopus and B. subtilis enzymes rapidly destroyed the NAD(P)ase.

Periodate specifically oxidizes diglycols and aminoglycols and is used in glycoprotein analysis (e.g., Spiro 1964 J. Biol. Chem. 239: 567). We performed the oxidations at both pH 4.0 and 7.5. At pH 4.0 NAD(P)ase was incubated at 25°C in the dark with 0.025 M sodium metaperiodate in 0.1 M sodium acetate buffer. At pH 7.5 NAD(P)ase was incubated at 25° C in the dork with 0.0125 M potassium periodate in 0.1 M Tris buffer. In both cases, the oxidation was stopped by mixing a 0.1 ml aliquot with 0.3 ml of 0.1 M sodium phosphate buffer pH 7.5 containing 0. 1 M ethylene glycol. Then NAD(P)ase was assayed by adding 0.1 ml NAD (4 mg/ml) as in Kaplan's standard assay. The results in Table II show that periodate rapidly inactivated NAD(P)ase at both pH's. These results ore consistent with the possibility that the carbohydrate portion of NAD(P)ore is required for its activity. The greater sensitivity at the higher pH is consistent with the aminosugars being more important than simple sugars; however, in view of the small amount of aminosugars in NAD(P)ase and the impurity of our enzyme preparation, this conclusion is tentative. These studies hove been terminated.

Table I

Lack of effect of phenylmethylsulfonyl fluoride on NAD(P)ase

duration of reaction (hours)	enzyme activity	
	control	+PMSF
0	0.49	0.50
0.25	0.45	0.55
2.5	0.54	0.50
6.5	0.50	0.56
14.5	0.52	0.49

NAD(P)ase incubated at 25° Cin0.02 M sodium phosphate buffer pH6.9, with or without 1 mM PMSF. Activity is the absorbance at 325 nm of NAD-CN in the Kaplan assay. Average of two trials.

Table II

Decrease in NAD(P)ase activity during periodate oxidation

duration of oxidation (hours)	NAD(P)ase activity			
	control pH 4.0	+ 0.025 M IO_4^- pH4.0	control pH 7.5	+ 0.0125 M IO_4^- pH7.5
0	0.35	0.37	0.34	0.36
0.5	0.34	0.19	0.37	0.04
1.0	0.36	0.14	0.32	0.02
3.0	0.33	0.06	0.29	0.01

Enzyme was incubated at 25° C in the dark at the pH shown, with or without periodate. Activity is the absorbance at 325 nm of NAD-CN in the Kaplan array. Average of two trials.

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