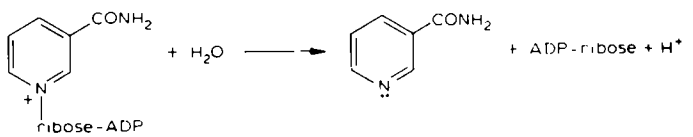
PRELIMINARY NOTE

BBA 61218

Kinetic properties of bull semen NAD glycohydrolase

NAD glycohydrolases (E.C. 3.2.2.5), commonly referred to as NADases, catalyze the hydrolytic cleavage of the cationic N-glycosidic bond of NAD:[1]



Such enzymes have been identified in a variety of mammalian tissues and in several microbial species; a summary of the properties of certain of the enzymes has been prepared[2]. In spite of the fact that the enzymes from pig brain and beef spleen, among others, have been widely employed for the synthesis of NAD analogs[3-10], particularly in the laboratories of KAPLAN[1,4,6,12] and ALIVISATOS[8,11], the kinetics of enzymatic NAD hydrolysis has been little explored. In this communication, the strikingly anomalous kinetic behavior of bull semen NAD glycohydrolase is reported.

Bull semen NAD glycohydrolase was purified according to the published procedure[11] and was stored at −20° in 0.25 M KCl, pH 7.5. Preparations employed for kinetic studies had specific activities near 1200 units/mg protein[11]; the basic features of the kinetic behavior, however, do not depend strongly on the state of purification of the enzyme. The course of the hydrolysis of NAD was followed titrimetrically on a Radiometer titrator, type TTTlc, equipped with a titrigraph SBR2, an all-glass burette, and a thermostated reaction vessel, usually maintained at 37°.

The course of the hydrolysis of NAD catalyzed by bull semen NAD glycohy-

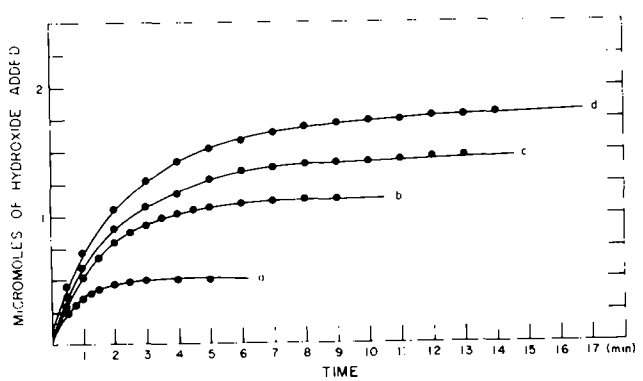


Fig. 1. Progress curves for the hydrolysis of NAD catalyzed by bull semen NAD glycohydrolase. The reaction was monitored by following the addition of hydroxide necessary to just neutralize the protons liberated in the hydrolysis reaction employing a Radiometer Titrimeter as a pH-stat. Each reaction was studied at pH 7.09 and at 36.5° in the presence of 0.25 M KCl in a total volume of 10 ml; enzyme concentration, 0.030 mg/ml. NAD concentrations are: (a) 0.05 mM; (b) 0.15 mM; (c) 0.20 mM; and (d) 0.40 mM.

drolase for four concentrations of substrate is shown in Fig. 1. Two points are of special note. First, at all concentrations of NAD employed, the kinetics are rather accurately first order (as revealed by plotting the data in the usual fashion for first-order kinetics). It is impossible to know if the concentrations employed in these experiments are saturating or not: the unusual kinetic behavior precludes determination of the value of K_m for NAD and that previously reported must be considered unreliable[11]. Suffice it to say that even at concentrations 50 times greater than the highest shown in Fig. 1, the same time course for the reaction is observed. Second, except for the lowest concentration of NAD employed, the reaction terminates prior to complete hydrolysis of NAD as estimated from the quantity of liberated protons. At an NAD concentration of 0.4 mM, less than half the stoichiometric number of

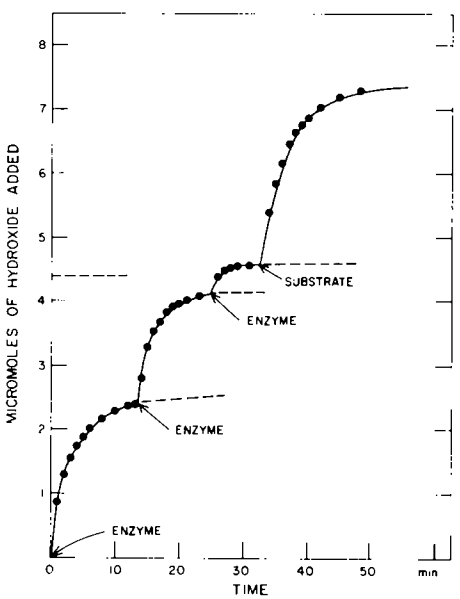


Fig. 2. Progress curves for the hydrolysis of NAD catalyzed by bull semen NAD glycohydrolase. The reaction was followed as described in Fig. 1. The initial reaction mixture contained 0.25 M KCl, 0.25 mM dithiothreitol, and 0.435 mM NAD in a total volume of 10 ml at pH 7.09 and 36.5°. At the indicated points, 0.5 ml of a 0.6 mg/ml solution of the enzyme was added and the reaction was followed until the rate of uptake of OH^- approached zero. The dotted line on the left indicates the theoretical OH^- uptake for total hydrolysis of the NAD originally present. Following complete hydrolysis of the NAD originally present, sufficient additional NAD was added at the indicated point to yield a solution 0.435 mM in this substrate.

protons is liberated. Addition of fresh substrate does not result in a further reaction. Dilution of the reaction mixture with buffer after termination of the reaction does not elicit further hydrolysis of unconsumed NAD. These points strongly suggest that termination of the reaction prior to complete substrate consumption is not the consequence of product inhibition nor of conversion of a portion of the substrate to an unreactive form. These points are clearly established by the data provided in Fig. 2. Successive additions of fresh enzyme to the reaction mixture eventually results in

complete consumption of the NAD. A subsequent addition of NAD elicits a reaction progress curve similar to the initial one. These results can only be accommodated by a process of inactivation of the enzyme in the presence of NAD. In the absence of this substrate, the enzyme is stable under the reaction conditions for several hours; the half-time for thermal denaturation of the protein under these conditions is 72 h.

The NAD-dependent enzyme inactivation is a reversible process as revealed by two lines of evidence. First, continued incubation of reaction mixtures that have reached the plateau stages indicated in Figs. 1 and 2 does result in a subsequent slow, zero-order reaction which continues until a quantitative yield of protons has been produced. This requires about 7–8 hours for the conditions of Fig. 1, reaction C. Second, dialysis of reaction mixtures containing high concentrations of NAD, so that the rapid phase of the reaction terminates prior to completion of the hydrolytic process, against buffer at 4° for 3.5 hours yields an enzyme preparation possessing approximately 80% of the original activity and characterized by the same type of kinetic behavior.

Two possibilities for the NAD-dependent inactivation process are evident: (i) a reversible transconformation reaction potentiated by the binding of substrate to enzyme; (ii) formation of a labile covalent bond between enzyme and a substrate fragment as a side reaction in the hydrolytic process leading to an inactive enzyme form. Efforts to distinguish between these possibilities are in progress.

Studies of the kinetics of hydrolysis of NAD catalyzed by the NAD glycohydrolase from pig brain[12] reveal normal behavior, in contrast to that noted above.

The financial support of the National Science Foundation and the Instituto Venezolano de Investigaciones Cientificas, Caracas, is gratefully acknowledged.

Department of Chemistry,
Indiana University,
Bloomington, Ind. 47401 (U.S.A.)

CONSTANTINE ZERVOS
RAFAEL APITZ
ALAN STAFFORD
E. H. CORDES

Centro de Bioquimica y Biofisica,
Instituto Venezolano de Investigaciones Cientificas,
Caracas (Venezuela)

1 N. O. KAPLAN, *Current Aspects of Biochemical Energetics*, Academic Press, New York, 1966, pp. 447–458.
2 D. STATHAKOS AND K. WALLENFELS, *Biochem. Z.*, 346 (1966) 89.
3 S. P. COLOWICK, J. VAN EYS AND J. H. PARK, in FLORKIN AND STOTZ, *Comprehensive Biochemistry*, Vol. 14, Elsevier, Amsterdam, 1966, p. 1.
4 L. J. ZATMAN, N. O. KAPLAN, S. P. COLOWICK AND M. M. CIOTTI, *J. Biol. Chem.*, 209 (1954) 453, 467.
5 B. M. ANDERSON AND N. O. KAPLAN, *J. Biol. Chem.*, 234 (1959) 1226.
6 B. M. ANDERSON, C. J. CIOTTI AND N. O. KAPLAN, *J. Biol. Chem.*, 234 (1959) 1219.
7 A. M. STEIN, J. K. LEE, C. D. ANDERSON AND B. M. ANDERSON, *Biochemistry*, 2 (1963) 1015.
8 S. G. A. ALIVISATOS, F. UNGAR, L. LUKACS AND L. LAMANTIA, *J. Biol. Chem.*, 235 (1960) 1742.
9 S. G. A. ALIVISATOS, L. LAMANTIA AND B. J. MATIJEVITCH, *Biochim. Biophys. Acta*, 58 (1962) 201.
10 S. G. A. ALIVISATOS, L. LAMANTIA AND B. J. MATIJEVITCH, *Biochim. Biophys. Acta*, 58 (1962) 209.
11 A. A. ABDEL-LATIF AND S. G. A. ALIVISATOS, *J. Biol. Chem.*, 237 (1962) 500.
12 H. G. WINDMUELLER AND N. O. KAPLAN, *Biochim. Biophys. Acta*, 56 (1962) 388.