tent of heparitin sulfate, while the relative percentage of fraction C remained unchanged, under the influence of the hormone evidently somehow prevented changes in the hydrodynamic properties of the gel filter. On withholding the hormone, against the background of an even greater fall in the heparitin sulfate level, the relative percentages of the other sulfated fractions also decreased, possibly in connection with a change in permeability due to the deposition of lipids in the aortic wall.

The results thus indicate that changes in permeability and deposition of lipids in the wall of the aorta, coupled with relative adrenal insufficiency caused by withholding hydrocortisone after its prolonged administration, are based on changes in the relative content of the various fractions of AMPS.

LITERATURE CITED

1. I. V. Ambrozas, in: Atherosclerosis and Membrane Permeability [in Russian], Leningrad (1974), pp. 36-38.
2. P. S. Khomulo and I. P. Zharova, Byull. Éksp. Biol. Med., No. 7, 17 (1972).
3. C. A. Antonopoulos, S. Gardell, J. A. Szismai, et al., Biochim. Biophys. Acta, 83, 1 (1964).
4. T. Bitter and H. Muir, Anal. Biochem., 4, 330 (1962).
5. S. T. Fu, R. J. Ho, C. B. Taylor, et al., Proc. Soc. Exp. Biol. Med., 142, 607 (1973).
6. R. Manthorpe, G. Helin, B. Kofod, et al., Acta Endocrinol. (Copenhagen), 77, 310 (1974).
7. R. N. Mullinger and G. Manley, J. Atheroscler. Res., 9, 108 (1969).
8. M. Stacey and S. Barker, Carbohydrates of Living Tissue [in Russian], Moscow (1965).
9. J. Svejcar and W. Robertson, Anal. Biochem., 18, 333 (1967).

SPONTANEOUS FLUCTUATIONS IN NAD-KINASE ACTIVITY OF RABBIT SKELETAL MUSCLES

I. D. Insarova, N. F. Belyaeva, and V. I. Telepneva

UDC 612.74.015.1:577.152.271

Spontaneous fluctuations in the time of activity of a 280-300-times purified preparation of NAD-kinase from rabbit skeletal muscles are described after its dilution. No fluctuations of activity were found in an unfrozen but undiluted preparation. After preincubation of the diluted enzyme with substrates (NAD and ATP) its activity did not fluctuate.

KEY WORDS: *NAD-kinase – dilution; fluctuations in activity; skeletal muscle.*

Previous investigations showed that NAD-kinase isolated from rabbit skeletal muscles has a complex quaternary structure and exists in solution as a system of dissociated oligomers, differing in their catalytic activity [1-3]. Interconversion of the oligomers takes place under the influence of various factors including the concentration of the reaction substrates and the protein concentration. The special features of the quaternary structure of NAD-kinase are also reflected in the complex kinetic behavior of the enzyme.

The results of an investigation of spontaneous fluctuations in enzyme activity of NAD-kinase in time are described in this paper.

Department of Biochemistry, Biological Faculty, Moscow State University, Moscow.

(Presented by Academician S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 8, pp. 957-959, August, 1976. Original article submitted January 9, 1976.

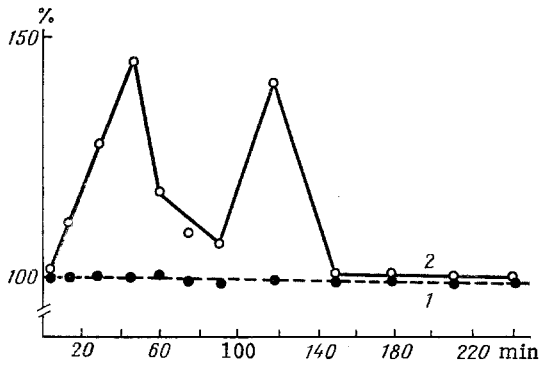

Fig. 1

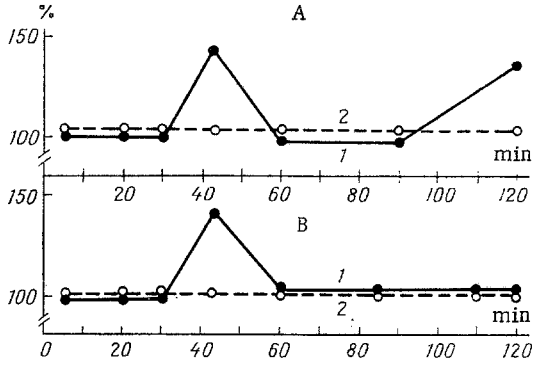

Fig. 2

Fig. 1. Specific activity of NAD-kinase as a function of keeping time of enzyme diluted after thawing. Dilution with 0.16 M Tris–HCl buffer, pH 7.3; keeping at 0°C: 1) without dilution, 2) diluted 1:3. Specific activity of enzyme measured immediately after dilution taken as 100%. Ordinate, specific activity (in %); abscissa, time after thawing of enzyme (in min).

Fig. 2. Effect of substrates on changes in specific activity of NAD-kinase with time after dilution. A: 1) Diluted 1:3; 2) diluted 1:3 in the presence of 3 μmoles NAD; B: 1) diluted 1:3 in the presence of 3 μmoles ATP, 2) diluted 1:3 in the presence of ATP and Mg^{2+} in the ratio 1:3. Remainder of legend as in Fig. 1.

EXPERIMENTAL METHOD

A preparation of NAD-kinase, purified 280-300 times, was obtained from the skeletal muscles of adult rabbits weighing 1.5-2 kg by the method described previously [1, 3]. NADP was synthesized in 1 ml of medium containing the following components (in μmoles): Tris–HCl (pH 7.3) 50, NAD 3, ATP 3, $MgCl_2$ 10, and 70-200 μg of the test protein. Incubation and determination of activity were carried out under the conditions described previously [1-3]. Protein was determined by the microbiuret method.

EXPERIMENTAL RESULTS

The preparation of NAD-kinase, kept at between –15 and –20°C, was thawed and diluted with 0.16 M Tris–HCl buffer, pH 7.3, and kept for a certain time at 0°C before addition to the incubation medium. To determine enzyme activity samples were taken immediately after dilution and thereafter at intervals of 5 min for 4 h. As Fig. 1 (curve 1) shows, keeping the unfrozen but undiluted enzyme at 0°C caused no change in its specific activity, whereas dilution led to the appearance of fluctuations in NAD-kinase activity (curve 2). Maximal activity was observed 45 and 120 min after dilution. The fluctuations of NAD-kinase activity were investigated in two preparations of the enzyme obtained in different years. Fluctuations of activity were observed in both preparations in all experiments (16 for the first and 12 for the second preparation). Although the samples tested differed a little as regards the position of the maximum and amplitude of the fluctuations of activity, similar changes could also be observed for each preparation during keeping. If K-phosphate buffer, pH 7.3, was used to dilute the enzyme instead of Tris–HCl the character of the relationship between specific activity and keeping time of the preparation at 0°C was unchanged.

Preincubation of the NAD-kinase with NAD and ATP (without Mg^{2+}) prevented the fluctuations in activity of the diluted preparation. As Fig. 2A (curve 2) shows, fluctuations of activity ceased completely after the addition of NAD alone. After addition of ATP to the sample the first maximum remained but the second disappeared (Fig. 2B, curve 1). Combined additions of ATP and Mg^{2+} abolished both maxima (Fig. 2B, curve 2).

Spontaneous fluctuations in enzyme activity after dilution were first described for ATPase activity and for the titer of SH groups of actin, myosin, and actomyosin [5-8]. The addition of ATP abolished the fluctuations and their amplitude varied during keeping of the preparations in the cold. Shnol' explains the fluctuations in ATPase activity of proteins by periodic synchronous conformational changes in their molecules. Shnol' considers that these fluctuations are caused by reversible conversions (oxidation) of protein SH groups.

Similar changes in enzyme activity with time have also been described for the creatine kinase of rabbit skeletal muscles [4]. In the opinion of Chetverikova and co-workers [4], conformational changes in the protein molecule synchronized throughout the volume of the solution also lie at the basis of these fluctuations.

It has been established that NAD-kinase from rabbit skeletal muscles is present in solution as an equilibrium system of dissociating oligomers which differ in activity [2, 3]. The possible cause of the fluctuations in NAD-kinase activity could be a spontaneous change in the degree of polymerization of the protein oligomer caused by dilution. Substrates in all probability fix the state of equilibrium existing at the time of their addition between the different oligomeric forms of the enzyme, as a result of which no change takes place in the specific activity of the enzyme when preincubated with substrates.

The authors are grateful to S. E. Shnol' for showing interest in the work and for actively participating in the discussion of the experimental results.

LITERATURE CITED

1. N. F. Belyaeva and V. I. Telepneva, Biokhimiya, 39, 975 (1974).
2. I. D. Insarova, A. N. Lebedev, and V. I. Telepneva, Dokl. Akad. Nauk SSSR, 219, 488 (1974).
3. V. I. Telepneva and I. D. Insarova, Dokl. Akad. Nauk SSSR, 218, 234 (1974).
4. E. P. Chetverikova, A. V. Krinskaya, N. A. Rozanova, et al., Biokhimiya, 35, 953 (1970).
5. S. É. Shnol', Vopr. Med. Khim., No. 6, 443 (1958).
6. S. É. Shnol', Biofizika, No. 2, 165 (1961).
7. S. É. Shnol', in: Molecular Biophysics [in Russian], Moscow (1965), p. 56.
8. S. É. Shnol', in: Oscillatory Processes in Biological Systems [in Russian], Moscow (1967), p. 22.