DYNAMICS OF DNA-BINDING ACTIVITY OF CYTOPLASMIC PROTEINS DURING AUTOPROTEOLYSIS

A. V. Zamotrinskii and A. I. Kuz'min

UDC 612.014:576.311].015:577.112].08

KEY WORDS: DNA-binding proteins; cytoplasm; proteolysis.

The cytoplasmic co-localization of certain nuclear proteins has now been established. These proteins include certain nonhistone proteins, but not histones [4]. It has been suggested that virtually all nonhistone proteins are in a state of equilibrium between nucleus and cytoplasm [3]. The possibility of a change in the subcellular localization of nuclear proteins also has been demonstrated, especially in the ontogeny of Xenopus [8] and Drosophila [6], in the form of stage-specific transitions of nuclear proteins located in the cytoplasm into the nucleus. The causes and mechanisms of the change in compartmentalization have so far received little study.

We have studied the possibility of the appearance of affinity for DNA in potential cytoplasmic DNa-binding proteins as a result of proteolysis, for we know that peptide intermediates of proteolysis differ from native precursors in their affinity for intracellular structures [2, 11].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino mice. DNA was isolated by chromatography of hydroxyapatite (Bio-Rad, USA) [5]. DNA-cellulose was prepared by freeze-drying and UV irradiation [13]. The liver cytosol was obtained by hypotonic shock (5 ml of 10 mM KCl, 10 mM Tris-HCl, pH 7.4, to 1 g of tissue, 15 min in an ice bath, after which the buffer was poured off), homogenization in a loose-fitting homogenizer, and centrifugation at 14,000 g for 10 min. By means of gel-filtration the proteins were transferred into incubation buffer (100 mM KCl, 25 mM NaCl, 2 mM EDTA, 10 mM tris-HCl, pH 7.4, at 37°C. Penicillin and streptomycin were added up to a concentration of 0.1 mg/ml of each to the cytosol with a protein concentration of 60 mg/ml, and 1-ml aliquots were incubated at 37°C, for a length of time indicated below in the text.

For incubation at acid pH values, proteins of the cytosol were transferred into 10 mM acetate buffer, pH 5.5, 37°C, containing 100 mM KCl, 25 mM NaCl, 2 mM EDTA, and 5 mM $MgCl_2$.

After incubation the buffer was changed in the aliquots to binding buffer (100 mM KCl, 25 mM NaCl, 2 mM EDTA, 10 mM Tris-HCl, pH 7.4, 4°C, 5% glycerol), and applied to identical plastic columns each containing 1 ml of DNA-cellulose. Cytoplasmic DNA-binding proteins were isolated as described for cell extract proteins [13]. The binding proteins were eluted with 0.6 mM NaCl, 2 mM EDTA, 10 mM Tris-HCl, pH 7.4, at 4°C, with 5% glycerol, and then with 2.5 M NaCl, 5.0 M urea, 10 mM Tris-HCl, pH 8.0, at 4°C. The first 2 ml of the eluate were collected and, after desalting, their protein concentration was determined from absorption at 206 nm [12] and by the method in [10].

Deslating and change of buffers were carried out by centrifugation on columns with Sephadex G-25 (Pharmacia, Sweden). Electrophoresis in 15% PAG was done by the method in [9]. The gels were stained with silver and scanned on an "Ultrascan" instrument.

EXPERIMENTAL RESULTS

At physiological values of ionic strength, and with application of 60 mg of cytoplasmic protein with 350 µg of immobilized DNA to the column, 10.8 µg of protein was eluted with a

Research Institute of Medical Genetics, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. V. Vasil'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 375-377, October, 1990. Original article submitted November 22, 1989.

 0007-4888/90/0010-1352$12.50

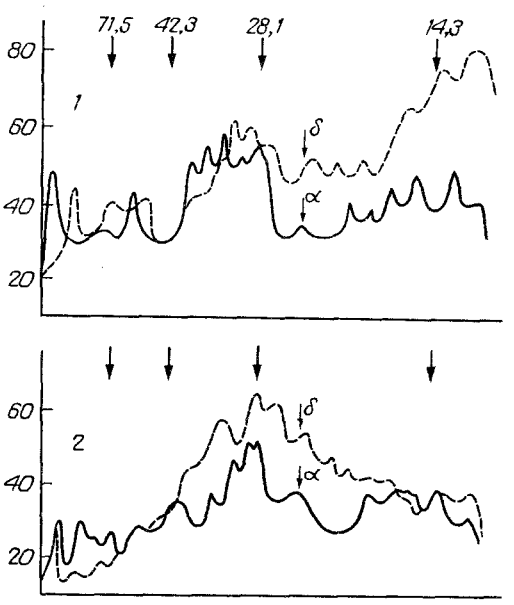

Fig. 1. Scans of mouse liver cytosol proteins, firmly (1) and weakly (2) bound with DNA: a) native cytosol, b) undergoing autoproteolysis for 150 min. Abscissa, molecular mass, kD (arrows show position of marker proteins); ordinate, optical density units.

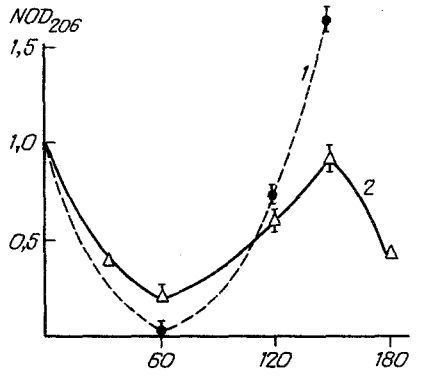

Fig. 2. Dynamics of DNA-binding activity of mouse liver cytosol during neutral autoproteolysis. Abscissa, incubation time at 37°C (in min); ordinate, optical density of eluates from DNA-cellulose. 1) Elution with 0.6 M NaCl, 2) with 2.5 M NaCl and 5 M urea.

high-salt buffer, 36% of which was eluted with 0.6 M NaCl (firmly and weakly bound proteins). Denaturing electrophoresis revealed heterogeneity of the protein components of both fractions as regards both molecular mass and quantitative representation. The weakly bound fraction consisted mainly of proteins with mol. mass of between 26 and 70 kD. Firmly bound proteins were more homogeneous and had a molecular mass of between 80 and 14 kD. The spectrum of DNA-binding proteins of the native cytosol is shown on the scans in Fig. 1.

Neutral autoproteolysis, continuing for 1 h, led to a decrease in the representation of all components of both fractions except a 26 kD labile-bound protein, whose concentration increased. With an increase in the incubation time the spectrum of DNA-binding proteins changed qualitatively: in the fraction of firmly bound proteins, components with mol. mass of under 25 kD, according to the results of denaturing electrophoresis, accumulated. The weakly bound fraction of proteolyzed cytosol was represented mainly by a group of 24-30 kD and 26 kD proteins. The spectrum of DNA-binding proteins undergoing autoproteolysis in the course of 2.5 h is shown on the scan in Fig. 1.

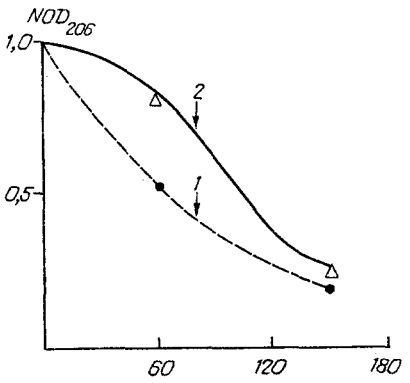

Fig. 3. Dynamics of DNA-binding activity of mouse liver cytosol during acid autoproteolysis. Legend as to Fig. 2.

To diminish the effect of breakdown products of native DNA-binding proteins on quantitative variations of DNA-binding activity during autoproteolysis, they were partially removed before the beginning of incubation. This was done by chromatography on DNA-Sephadex [2]. After two recirculations of 6 ml of cytosol through the carrier containing 5.7 mg DNA, about 40% of the original amount of DNA-binding proteins remained in the cytosol. Further experiments were carried out on cytosol fractionated in that way.

The DNA-binding activity of the low-speed mouse liver cytosol during neutral autopyrolysis had a marked biphasic character. The maximal amount of labile-bound proteins of the proteolyzed cytosol was observed after 3 h of incubation, the firmly bound proteins at 2.5 h. Quantitative changes in the content of DNA-binding proteins are illustrated in Fig. 1.

Since during repeated experiments the quantity of immobilized DNA decreased [13], values of optical density of the eluates were normalized relative to the corresponding values of the unincubated samples. For points with reliable values, three chromatographic experiments were obtained on two samples of DNA-cellulose.

The dynamics of DNA-binding activity was unchanged after preliminary passage of the cytosol through columns each containing 6 ml of cellulose.

The protease inhibitor $Na_2S_2O_5$ in a concentration of 10 mM prevented the appearance of the second peak of DNA-binding proteins and led to stabilization of the content of firmly bound proteins and to a steady decrease in the amount of labile-bound proteins (after 2.5 h of incubation there remained 95 and 12% of them respectively). The addition of ATP up to 10 mM to the cytosol before the beginning of incubation had a similar effect (after 2.5 h no labile-bound proteins could be detected, and only 40% of the firmly bound proteins remained).

Incubation under optimal conditions for activation of the lysosomal component of proteolysis did not lead to the appearance of the second peak of DNA-binding proteins (Fig. 2).

The polphasic nature of the content of cytoplasmic DNA-binding proteins also was observed in the liver of CBA mice and in Ehrlich's ascites tumor cells. For both of these, the time of appearance and the amplitude of the second peak of DNa-binding activity are dependent on the conditions of growth of the tumor and, in particular, on the time after transplantation of the cells. Tumor cells also possess a more than fourfold increase in the content of labile-bound proteins compared with firmly-bound (for liver cytosol their ratio was 0.59 ± 0.017, for the cytosol of Ehrlich's cells 2.54 ± 0.25; mean data from three experiments) (Fig. 3).

Thus mouse liver cytosol contains proteins which, as a result of neutral autoproteolysis, acquire affinity for DNA. Since affinity for intranuclear structures is essential for the nuclear localization of a protein [7], proteolysis may be the cause of the change in compartmentalization of certain cytoplasmic proteins.

LITERATURE CITED

1. A. I. Fedorov and O. O. Favorova, Mol. Biol., 22, No. 1, 267 (1988).
2. J. S. Bearden, J. Biochem. Biophys. Meth., 2, 37 (1980).

3. M. Bustin and N. K. Neihart, Cell, 50, No. 1, 181 (1979).
4. D. E. Commings and D. C. Harris, J. Cell Biol., 70, No. 2, 440 (1976).
5. Y. M. T. Cseko, J. Stone, and R. Sederoff, Biochim. Biophys. Acta, 565, No. 2, 253 (1979).
6. R. Dequin, H. Saumweber, and J. W. Sedat, Dev. Biol., 104, No. 1, 37 (1984).
7. C. Dingwall and R. A. Lasket, Annu. Rev. Cell. Biol., 2, 367 (1986).
8. C. Dreyer and P. Haysen, Dev. Biol., 100, No. 26, 412 (1983).
9. U. K. Laemmli, Nature, 227, 680 (1970).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, No. 1, 265 (1951).
11. N. T. Neff, L. Bourret, P. Miao, and J. F. Dice, J. Cell Biol., 91, No. 10, 181 (1981).
12. R. K. Scopes, Anal. BIochem., 59, No. 1, 227 (1974).
13. G. H. Stein, Meth. Cell. Biol., 17, No. 2, 271 (1978).

EFFECT OF ELEMENTARY SULFUR ON ADENYLATE KINASE ACTIVITY AND WORKING OF THE ISOLATED RABBIT HEART

A. I. Dagys, K. T. Vitkevičius,
G. A. Balčiunas, V. I. Gendviliene,
P. P. Džeja, and A. I. Toleikis

UDC 612.17.014.46:546.22].085

KEY WORDS: heart, sulfur, adenylate kinase, vasodilatation.

Elementary sulfur is used in industry, agriculture, and medicine. Besides as an external application, intravenous injections of sulfur in peach oil also are used in medicine for pyrogenic treatment [1]. The mechanism of its action has not been explained. It has recently been shown [2] that sulfur inactivates adenylate kinase (AK), one of the most important enzymes in energy metabolism of cells. It has also been shown [3] that sulfur has a moderate uncoupling and inhibitory effect on respiration of the cardiac mitochondria, and in combination with dithiothreitol, it inhibits adenine-nucleotide translocase. The writers previously postulated [4] a role for adenylate kinase in intracellular processes of energy transport and in the energy supply for myofibrillary contractions.

The aim of this investigation was to study the possibility that sulfur may inhibit AK in living cells and the effect of sulfur itself on the work of the isolated rabbit heart and on the coronary perfusion flow. Temporary inhibition of AK activity may also be an essential measure for preventing degradation of adenine nucleotides during tissue ischemia, thereby facilitating restoration of the working capacity of the organs in the postischemic period [5].

EXPERIMENTAL METHOD

The isolated rabbit heart was perfused by Langendorff's method under isometric conditions with modified Tyrode solution (144 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 10 mM Tris-HCl, 10 mM glucose, pH 7.4), saturated with oxygen. Throughout this period the amplitude and frequency of the cardiac contractions were recorded and the velocity of the coronary flow of perfusion fluid measured. The amplitude of the cardiac contractions was measured by means of a 6MKh IC mechanotron. Activity of AK isozymes was measured as described previously [6]. Mitochondria were isolated from the rabbit heart as in [7].

EXPERIMENTAL RESULTS

The mechanism of action of sulfur and its effect on activity of individual AK isozymes still remained unknown.

Laboratory of Metabolism, Central Research Laboratory, Kaunas Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR J. J. Bredikis.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 377-379, October, 1990. Original article submitted December 30, 1989.

