Journal of Solid-Phase Biochemistry, Vol. 5, No. 1, 1980

METHOD OF STABILIZATION OF SH-ENZYMES: STABILIZATION OF FORMATE DEHYDROGENASE AND ALCOHOL DEHYDROGENASE BY POLYCATIONS

M. M. DIKOV, A. P. OSIPOV, A. M. EGOROV, A. Y. KARULIN, M. I. MUSTAFAYEV, and V. A. KABANOV

Chemical Department, M.V. Lomonosov Moscow State University, Moscow 117234, USSR

Accepted August 8, 1979

Dehydrogenases capable of oxidation can be stabilized by complexation with polyelectrolytes. These polymers prevent the oxidation of SH groups by the formation of a highly charged microenvironment.

Practical application of many enzymes is limited by their SH-groups' lability toward oxidation catalyzed by metal ions. To solve this problem we suggest an approach based on the utilization of polyelectrolytes on the basis of quaternized polyvinylpyridine, which is bound to globular proteins, forming an "individual" microenvironment for the enzyme molecule that prevents the penetration of metal ions. The following polymers have been synthesized: polycation (PC), amphoteric polyelectrolyte (AP), and polymer containing anionic groups (PA). These last are poly-4-vinyl-*n*-ethylpyridine bromide of 40×10^3 (PC–40) and 200×10^3 (PC–200) molecular weight, copolymers of 4-vinyl-pyridine with 4-vinyl-*N*-ethylpyridine bromide and 4-vinyl-*N*-acetylpyridine bromide (degrees of ethyl bromide and bromacetic acid alkylation are 21 and 56%, respectively), and 4-vinylpyridine with 4-vinyl-*N*-acetyl-pyridine bromide (degree of bromacetic acid alkylation is 50%) of 100×10^3 molecular weight. Formation of complexes by such polymers with one or several protein globules surrounded by the polymer shell has been studied (1). In this work the influence of polyelectrolytes on stability and activity of formate dehydrogenase (EC 1.2.1.2; FDH) from methylotrophic bacteria, strain no. 1, and alchohol dehydrogenase (EC 1.1.1.1; ADH; "Reanal," Hungary) from horse liver has been studied.

The molecule of FDH consists of two identical sub-units and contains 12 SH-groups (2,3). Inactivation of the enzyme at 37°C is accompanied by a decrease of the total number of SH-groups titrated with 5,5′-dithyobis

0146-0641/80/0300-0001$03.00/0

TABLE 1. **Titration of SH-Groups on FDH and ADH**

Formate dehydrogenase			Alcohol dehydrogenase		
Time (h)	Native FDH, total SH-groups (%)	Complex FDH–PC–40, total SH-groups (%)	Time (h)	Native ADH, total SH-groups (%)	Complex ADH–PC–40 total SH-groups (%)
20	92	100	10	96	100
30	83	98	20	92	99
35	75	98	30	89	98
45	51	98	40	67	96
65	30	97	50	14	94

(2-nitrobenzoic acid) in 8 M urea and occurs only in the presence of metal ions (Cu^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}) (Table 1). Ethylendiaminetetraacetic acid (EDTA) has a considerable stabilizing effect under these conditions (4,5). The inactivation of ADH at 37°C is also associated with oxidation of its SH-groups (Table 1). The formation of complexes of FDH and the polyelectrolytes was performed by ultracentrifugation or double immunodiffusion of rabbit antiserum against FDH and ADH. Addition of the enzyme to PC solution decreases the peak of polymer on the sedimentation curve. The peak completely disappears when the FDH-PC molar ratio is equal to 1:1 and 5:1 for PC–40 and PC–200, respectively. The intensity of the precipitation lines decreases with the increase of polymer concentration while the solution of FDH-polyelectrolyte complexes is titrated with rabbit antiserum against FDH. The existence of ADH-polycation complexes was proved by similar ultracentrifugation experiments.

According to assumptions in (1,4) the polymer forms a shell around one or several protein globules. Formation of the complex does not lead to any considerable changes in enzyme structure, for it does not change the spectral and fluorescence properties of enzymes. V_{max} remains constant, while K_m for formate and NAD^+ changes insignificantly for FDH. Polyelectrolytes do not influence the initial activity of ADH.

At 37°C the effect of FDH stabilization by polycation increases with the increase of positive charge of the polycation (Fig. 1). The maximal stabilizing effect determined by comparison of the first-order rate constants of monomolecular inactivation reaches 450 at a conversion extent of 50%. Negatively charged groups (AC) decrease the stabilizing effect of the polyelectrolyte. The increase in the negative polymer (PA) charge results in enzyme destabilization.

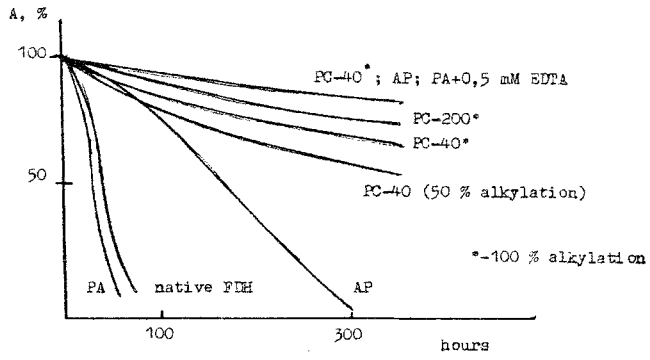

FIG. 1. Stabilization of FDH by polycationic polymers.

In the presence of 0.5 mM EDTA, all the FDH-polymer complexes have the same stability despite the differences in the charge of the polymer. Hence alteration of the polymer charge influences only the rate of oxidation of the enzyme SH-groups and does not influence conformational stability of the complex. Complexes of ADH with polycations show higher stability in comparison with the negative enzyme (Fig. 2). The introduction of negatively charged groups into the polymer structure decreases the stability of the complexes.

The mechanism of stabilization of dehydrogenase is thought to be the following. According to the data on the structure of complexes, polycation molecule covers the protein globule and forms around it a positively charged shell. Electrostatic repulsion of positively charged metal ions by the polymer shell prevents their penetration of the protein molecule. As a result, the catalytic oxidation of SH-groups with oxygen does not occur, and a principle

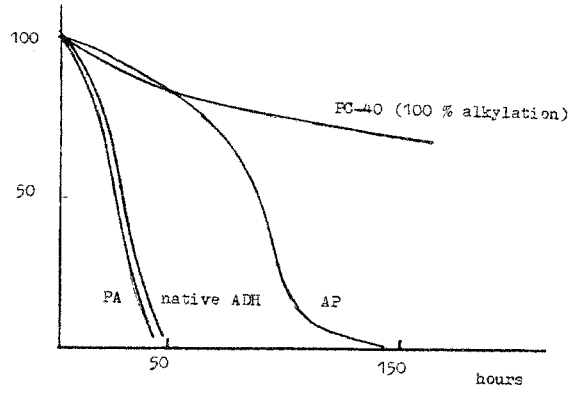

FIG. 2. Stabilization of ADH by polycationic polymers.

for removing the metal ions from the microenvironment of the protein globule is realized.

The destabilizing effect of polycations can possibly be explained by the fact that the negatively charged shell concentrates metal ions in the microenvironment of the enzyme. This leads to a more effective oxidation of the SH-groups. The method based on the complex formation with polycations can be expanded to other enzymes liable to oxidation of SH-groups. Thus we consider that the formation of a positively charged shell around the protein globule by polycations on the basis of quaternized 4-vinylpyridine is one of the general methods for stabilization of an enzyme, the catalytic activity of which is affected by oxidation of SH-groups catalyzed by metal ions.

REFERENCES

1. KABANOV, V. A., EVDAKOV, V. P., MUSTAFAYEV, M. I., and ANTIPINA, A. D. (1977) Molek. Biol. 11 ; 582.
2. RODIONOV, YU. V., AVILOVA, T. V., ZAKHAROVA, E. N., PLATONENKOVA, L. S., EGOROV, A. M., and BEREZIN, I. V. (1977) Biokhimiya 42 ; 1896.
3. POPOV, V. O., and EGOROV, A. M. Biokhimiya (1979) 44 ; 207.
4. DIKOV, M. M., OSIPOV, A. P., EGOROV, A. M., BEREZIN, I. V., MUSTAFAYEV, M. I., KIRSCH, YU. E., and KABANOV, V. A. (1979) Dokl. AN SSSR (Russia), in press.
5. DIKOV, M. M., KARULIN, A. Y., OSIPOV, A. P., and EGOROV, A. M. (1979) Bioorg. Khimiya (Russia) 5(8).