Effect of Pentosan Polysulfate on the Thermal Stability of Yeast Alcohol Dehydrogenase

M. MOLNÁROVÁ, H. PAULÍKOVÁ and D. PODHRADSKÝ

Department of Biochemistry Faculty of Sciences University of P. J. Šafárik, Moyzesova 11, SK-040 11 Košice

The aim of this study was to determine the effect of pentosan polysulphate on the thermostability of alcohol dehydrogenase. Spectral analyses showed that this compound is able to increase thermostability of the enzyme and entirely eliminate thermal aggregation of YADH. These findings indicate that this heparinoid can not only modulate the enzyme activity (it has been presented in our recent paper) but they also can prevent protein thermal denaturation.

Alcohol dehydrogensase (EC 1.1.1.1) from yeast (YADH) is a medium-chain zinc-containing alcohol dehydrogenase. Several isozymes of YADH from cytosol and mitochondria have been characterised [1,2]. The thermal stabilisation of those ADHs that are normally thermolabile, by addition of structure stabilisation molecules, should be useful for wide application in biological and industrial field. Some of the polyanions have high affinity to proteins, e.g. polysulphated polysacharides, heparin being the most common. It hase been shown in our laboratory that some of the polysulfated polysacharides can interact with YADH [3]. We implied that the stabiliy of YADH could be changed after binding derivatives of polysulfated polysacharides. In the present study, the effect pentosan polysulfate (PS) on the thermostability of YADH has been investigated.

Materials. Yeast alcohol dehydrogenase and pentosan polysulfate were purchased from Sigma Chemical Company (St.Louis, MO, U.S.A). Pentosan polysulfate was sodium salt with average molecular weight of 3 000. All other reagents were from Aldrich Chemie (Steinheim, Germany).

Spectral measurements of thermal denaturation. Thermal stability was monitored in the range of the temperatures from 18 °C to 90 °C. The temperature of all compartments was increased continuously and ultraviolet difference spectra of YADH were measured on Shimadzu UV – 3 000 after every increase of temperature about 2 °C. The stock solution of YADH (12.2 μM) was prepared by dilution of the enzyme in 50 mM phosphate buffer, pH 6.5, 7.0 and 8.0. The final concentration of the enzyme in cuvette was 81 nM. Maximal spectral changes at 276 nm were taken as 100% denaturation. T_d value is the temperature at which 50% denaturation of protein takes place.

Thermal stability of YADHI (the main isozyme of the enzyme obtained from Sigma) was investigated by spectral analysis. The effect of PS on thermal stability of YADHI were compared with thermostabilization effect of phosphate. The obtained results are summarised in Table 1. Heat denaturation of YADH was influenced by pH, the differences between T_d at pH 6.5 and 8.0 were 15.3 °C in 1.5 mM phosphate buffer and 15.1 °C in 150 mM phosphate buffer. The thermostability of YADHI depends mainly on the pH value. YADHI has cysteine residues (cys^{277}) in the interface domains. The change of oxidised/reduced state above mentioned cysteine residues could influence the dissociation of the protomers [4]. Thermostability was increases at pH 8.0 when the creation of the S-S bridges is facilitated.

Table 1. Dependence of T_d of YADH on pH, phosphate buffer concentration and the presence of PS (8.79 μM).

	pH	T_m[°C] Concentration of phosphate buffer	
		1.5 mM	150
YADH	8.0	63.5	65.0
YADH	6.5	48.2	49.9
YADH+PS	6.5	54.4	60.2

The effect of PS (8.79 μM) on the thermal stability of YADH was estimated. PS increased T_d (Table 1) about 6.2 °C in 1.5 mM phosphate buffer and about 10.3 °C in 150 mM buffer. PS could to be potent stabiliser of YADH. This considerable thermoprotection effect of PS on YADH may be explained as the prevention of thermal aggregation of the protein. PS is able to prevent aggregation even at 75 °C (the result is not shown). The results of spectral measurements support our concept that the heparinoic increases the solution stability of YADH.

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