PROTEOLYTIC RESISTANCE AND THERMOSTABILITY OF CATALASE AND HISTIDINE DECARBOXYLASE FROM *Micrococcus* sp. n.

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One stage in the investigation of the structural and functional properties of enzymes is establishment of their degree of resistance under conditions of limited proteolysis and determination of the parameters of thermal inactivation [6]. The aim of this investigation was to study these properties of homogeneous heme-containing catalase and of crystalline homogeneous histidine decarboxylase (HDC) from *Micrococcus* sp. n.

EXPERIMENTAL METHOD

Preparations of HDC and catalase with specific activities of 0.45 and 940 μg-atoms/mg, respectively, were isolated from a culture of *Micrococcus* sp. n. by the method described previously [3]. Activity of the enzyme was determined manometrically in a Warburg apparatus [2], using 0.2 M K-phosphate buffer, pH 5.5 (for HDC) and pH 7.5 (for catalase). Protein was determined by the method in [5]. Proteolytic resistance (PR) of catalase and HDC was determined by measuring activity after limited proteolysis with trypsin (from Sigma, USA). The reaction was carried out at 37°C in 0.1 M K-phosphate buffer, pH 7.8, with different proportions of trypsin and substrate by weight. Proteolysis was stopped by the addition of phenylmethanesulfonylfluoride (from Fluka, Switzerland).

Thermal resistance of HDC and catalase was determined by measuring activity after incubation of the enzymes (40 μg) in 0.1 ml of 0.1 M K-phosphate buffer, at different pH values, for 5 min. Electrophoresis in 7.5% polyacrylamide gel was carried out in an apparatus from Pharmacia Fine Chemicals (Sweden) [4].

EXPERIMENTAL RESULTS

Investigation of PR of heme-containing catalase and of HDC showed that both enzymes are virtually completely resistant to the action of trypsin if the protease is used in the con-

TABLE 1. Thermal Inactivation of Catalase and HDC at Different Temperatures and pH Values

pH	Enzyme	Temperature, °C				
		42	52	62	72	82
5,55	Catalase	82	79	70	55	43
	HDC	100	99	96	92	10
6,45	Catalase	81	78	73	60	45
	HDC	100	95	92	91	8
7,45	Catalase	100	100	100	81	60
	HDC	100	97	95	32	10

Legend. Values of residual activity (in %) of enzymes after incubation for 5 min at corresponding pH and temperature are given (mean results of 3 experiments).

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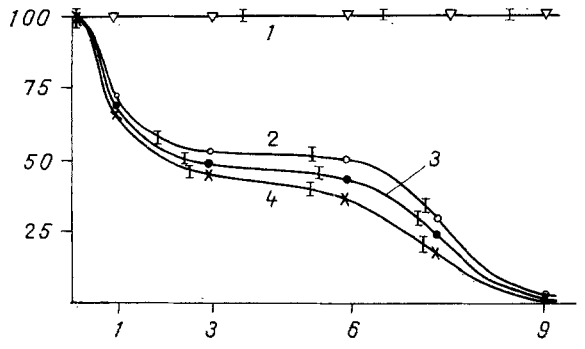

Fig. 1

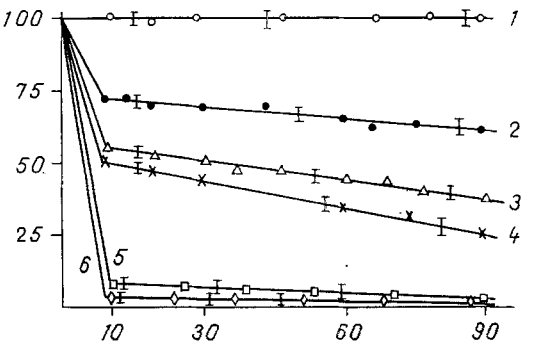

Fig. 2

Fig. 1. Proteolytic resistance of catalase and HDC depending on duration of hydrolysis and protein/protease ratio. 1) Catalase (hydrolysis for 30 min); 2) HDC (hydrolysis for 10 min); 3) HDC (30 min); 4) HDC (60 min). Abscissa, ratio trypsin/enzyme by weight; ordinate, residual activity (in %).

Fig. 2. Proteolytic inactivation of HDC during limited proteolysis by trypsin. 1-6) Ratio HDC/trypsin by weight 50:1 or 10:1, 1:1, 1:3, 1:6, 1:10, and 1:20, respectively. Abscissa, duration of hydrolysis (in min); ordinate, residual HDC activity (in %).

centrations that are usually used for investigation of PR of different enzymes during limited proteolysis: Inactivation did not take place if the protein/protease ratio exceeded 10:1. With an increase in protease concentration, substantial differences began to appear in PR of catalase and HDC (Fig. 1). Trypsin, even in a tenfold excess, and with hydrolysis for 60 min, was unable to hydrolyze the catalase molecule, so that either the catalytic activity was reduced or additional zones with different electrophoretic mobility appeared, evidence of fragmentation of the protein. Since, as the results of a study of amino acid composition showed, the polypeptide chains of the catalase subunits could not contain regions suitable for attack by trypsin, the native state of active catalase must be regarded as one in which these regions are masked in the depth of the protein globule or are securely screened by other functional groups.

Unlike catalase, in the case of HDC the degree of inactivation showed marked dependence on the duration of hydrolysis and protease concentration (Fig. 2). During the first 9 min of hydrolysis there was a sudden stepwise drop of activity, followed by relatively slow linear inactivation of the enzyme. This fact probably indicates that removal of the N-terminal lysine-rich fragment of the HDC β-chain takes place initially [1], followed by relatively uniform degradation of the whole molecule.

Experiments on thermal inactivation of catalase and HDC revealed a high degree of resistance of the enzymes to temperature denaturation (Table 1). When homogeneous preparations of enzymes were used, irreversible thermal inactivation after incubation at a temperature of over 65°C was 15-20% higher than during work with partially purified enzymes. Investigation of thermostability at different pH values showed that 70-80% of activity of catalase and HDC remained after incubation for 5 min at 72°C only if that incubation is carried out at pH close to the pH optimum for activity of the enzyme: pH 7.45 for catalase, pH 5.55 for HDC.

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