MULTIFUNCTIONALITY OF YEAST GLUTATHIONE REDUCTASE

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Glutathione reductase (EC 1.6.4.2) promotes various NADPH-linked one-electron transfer and two-electron transfer reactions. These reactions are, likewise, facilitated by the free reduced FAD (1). Studies with heart lipoamide dehydrogenase reveal the modulation of the flavin reactivities by the apoenzyme structure (2). Since the complete amino acid sequence (3) and the three-dimensional structure (4) of glutathione reductase from erythrocyte are known, studies of glutathione reductase may further delineate the role of protein structures in regulating multi-functional activities of flavoenzymes.

The purified glutathione reductase from yeast exists in a single molecular form with an isoelectric pH of 6.08 (Figure 1). In addition to

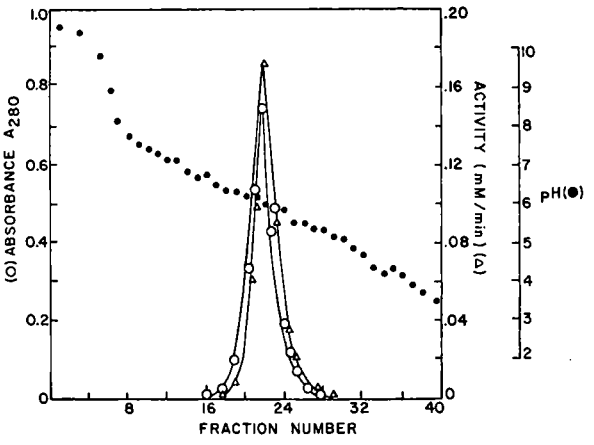

Fig. 1. Isoelectric focusing of yeast glutathione reductase

Flavins and Flavoproteins

its physiological reductase (RDase) activity, the yeast enzyme also displays nicotinamide nucleotide transhydrogenase (THase), inorganic electron transferase (ETase) and quinone diaphorase (DPase) activities (Table 1).

Table 1

Kinetic parameters (asymptotic) for reactions catalyzed by yeast glutathione reductase at pH 7.0

Multifunctional activities	V ($\mu M\ min^{-1}$)	K_a (μM)	K_b (μM)	K_{ia} (μM)	V/E_t (min)
RDase	148	174	198	49.8	20.4×10^3
THase	1.89	6.83	49.4	0	152
ETase	47.3	154	445	0.68	4.5×10^3
DPase	9.50	106	297	44.4	909

Chemical modifications were carried out to correlate the multifunctional activities of glutathione reductase with critical amino acid residues (Table 2). Reductive carboxymethylation which monomerized the enzyme preferentially inactivated the RDase activity. The active site of glutathione reductase consists of flavin coenzyme, the redox active disulfide, Cys 58 and Cys 63 (5,6) and the catalytically competent His 467' (7). These residues are obligatory for the RDase activity as demonstrated by the elimination of the RDase activity by arsenite chelation which blocked the active-site disulfide, and sensitized photo-oxidation of histidine residues. X-Ray crystallographic study of erythrocyte glutathione reductase (8) reveals the localization of Tyr 197 in the binding pocket of NADP(H) and pairs of salt-bridges, Lys 66-Glu 201 and Arg 291-Asp 331, surrounding the active-site flavin. Treatment of the yeast enzyme with tetranitromethane which nitrated 1.2 $\pm$ 0.2 tyrosine residue per FAD abolished all multifunctional activities. Amidination with imidoesters which derivatized lysine residues, did not affect any of the multifunctional activities. However, amidation of carboxylic groups with ethylene diamine which reversed the charge

Table 2

Variation in Multifunctional Activities by Chemical Modifications

Enzyme derivatives	Effect	% Activity of Control			
		RDase	THase	ETase	DPase
E_{rcM}	Monomer	0	0.15	24	5.6×10^3
$E_{h\nu}$	His	2.0	11	74	119
E_{As}	-S-S-	0	41	73	372
$E_{NH_3^+}$	COOH	19	200	724	112
E_{CH_3}	COOH	41	155	344	110
$E_{SO_3^-}$	COOH	38	133	198	108
E_{TNM}	Tyr	4.0	16	9	6
E_{ma}	Lys	100	104	106	91

Chemical modifications; reductive carboxymethylation (11) with iodoacetate (E_{rcM}), photooxidation (2) with rose bengal ($E_{h\nu}$), chelation (13) with arsenite (E_{As}), sensitized amidation (12) with ethylene diamine ($E_{NH_3^+}$), propylamine (E_{CH_3}) and taurine ($E_{SO_3^-}$), nitration (14) with tetranitromethane (E_{TNM}) and amidination (15) with methyl acetimidate (E_{ma}) were carried out as described in the literatures.

character, specifically enhanced the ETase activity.

An extensive sequence homology of the active site (6,9), albeit a detailed structural difference (Table 3), exists between lipoamide dehydrogenase and glutathione reductase (10). Their apoenzymes display similar structural perturbation effects on the multifunctional activities resulted from the reduced flavin. The nonphysiological ETase and DPase activities are suppressed by carboxylic groups and dimeric structure respectively. While the physiological RDase activity which requires the dimeric structure and the redox active disulfide is aided by the active-

site histidine. An intermediate effect on the THase is generally observed.

Table 3

Empirical structural parameters for the redox-active disulfide containing octadecapeptides of heart lipoamide dehydrogenase and yeast glutathione reductase

Empirical parameter (Ave. per residue)	Lipoamide dehydrogenase	Glutathione reductase
P_α	0.92	0.94
P_β	1.02	1.13
Mol. vol. (nm) x 10^{-3}	62.4	65.2
Acc. surface area (nm^2)	0.376	0.375
Hydropathy index	0.61	1.04
Free energy (Kcal mol^{-1})	1.17	1.31

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