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The role of essential –SH groups of yeast alcohol dehydrogenase

The NADH-dependent dehydrogenases are assembled from subunits with molecular weights ranging from 30 000 to 50 000[1] Each of these subunits has a single coenzyme binding site[1] and a single essential –SH group[2], both of which are considered to be in the active center The role of such an essential –SH group has been interpreted in at least two different ways, it might either (a) bind the coenzyme to the active center or (b) regulate the structure of this center without binding the coenzyme Previous work[3] supports the first possibility by indicating that the essential –SH groups of yeast and liver alcohol dehydrogenases serve the function of binding the nicotinamidic moiety of the coenzyme MULLER HILL AND WALLENFELS[4] reached different conclusions They studied the role of –SH groups of yeast alcohol dehydrogenase (alcohol NAD$^+$ oxidoreductase, EC 1 1 1 1) using *p*-chloromercuribenzoate (PCMB) as an –SH group reagent[4] The capacity of yeast alcohol dehydrogenase to bind NADH was found decreased after reaction with PCMB However, since PCMB has not been shown to be a specific reagent for the essential –SH groups of yeast alcohol dehydrogenase, this study did not clarify the role of the essential –SH groups. Furthermore, these authors were not able to make reliable NADH binding capacity determinations by using ultracentrifugation[4]. Therefore, in the present study we have investigated again the role of essential –SH groups of yeast alcohol dehydrogenase. This enzyme was reacted with iodoacetamide, a specific essential –SH groups reagent of this enzyme[5] The NADH binding capacity of the enzyme was measured by our modification[1] of the technique of HUMMEL AND DREYER[6]

Yeast alcohol dehydrogenase (Boehringer) suspended in ammonium sulphate was dialyzed against 67 mM phosphate buffer (pH 7 2) The dialyzed enzyme solution was centrifuged to remove some insoluble material, and the protein content[7] and enzymatic activity[8] were measured Part of this solution was used for control experiments, another part was incubated at 25° with iodoacetamide or *N*-ethylmaleimide (10 equiv /mole enzyme) or with maleimide (5 equiv /mole enzyme) When the enzyme inactivation reached the desired extent, reduced glutathione was added to the reaction mixture (5 equiv/mole of –SH groups reagent) to prevent further enzymatic –SH group modification The method used to measure NADH binding capacity was as previously described[1] Native enzyme (0 06 μmole) and iodoacetamide-inhibited enzyme (94%) were filtered through a Sephadex column, calibrated for molecular weight[9]

The samples of yeast alcohol dehydrogenase were selected for the inactivation experiments reported herein only after determining the turnover number, the diagram of elution from a Sephadex G-50 column and the NADH binding capacity Great heterogeneity was found among all the samples examined by these criteria. The values for the turnover number ranged from 9000 to 35 000 Several enzyme samples, when filtered through Sephadex G-50 columns, showed two peaks of protein. Only the first peak was enzymatically active

Fig 1 shows that for preparations having a single peak on Sephadex, there is

Abbreviation PCMB, *p*-chloromercuribenzoate

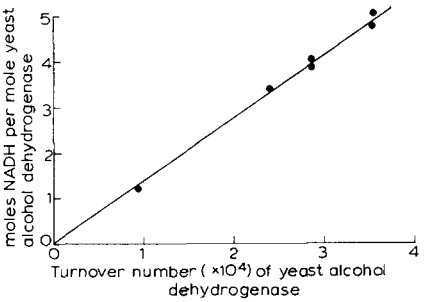

Fig 1 Plot of NADH binding capacity against the turnover of yeast alcohol dehydrogenase preparations

a linear positive relationship between turnover number and NADH binding capacity In the inactivation experiments reported, only enzyme samples with a turnover number of at least 24 000 showing a single peak of protein, which was active, on Sephadex G-50 chromatography were used

Table I shows the effect of three different –SH group reagents on NADH binding capacity of yeast alcohol dehydrogenase After partial and total inactivation of the enzyme by iodoacetamide, the enzyme still binds 63 and 71%, respectively (Expts 2 and 3), of the amount of coenzyme bound by the native protein In another experiment no appreciable difference in NADH binding to fully or 50% inactivated enzyme was detected (Expt 1 in Table I) Maleimide, which partially precipitated

TABLE I

THE NADH BINDING CAPACITY OF YEAST ALCOHOL DEHYDROGENASE AFTER INACTIVATION WITH SULFHYDRYL REAGENTS

Expt No	*Inhibitor*	*Yeast alchol dehydrogenase % Inhibition*	*Turnover number of yeast alcohol dehydrogenase before inactivation*	*Moles NADH per mole enzyme**
1	Iodoacetamide	Control	35 000	4 8
		50	35 000	3 4
		100	35 000	3 0
2	Iodoacetamide	Control	35 000	5 1
		95	35 000	3 6
3	Iodoacetamide	Control	28 000	4 0
		97	28 000	2 5
4	Iodoacetamide	Control	24 000	3 3
		96	24 000	2 6
		96	24 000	2 8
		96 5	24 000	2 8
5	Maleimide	Control	28 000	3 8
		8	24 000	3 6
6	Maleimide	Control	35 000	4 8
		97	31 000	1 3
7	*N*-Ethylmaleimide	Control	30 000	4 0
		90	30 000	2 0

* A molecular weight of 150 000 (ref 11) was used

the enzyme during the incubation, and *N*-ethylmaleimide reduced the NADH binding capacity to 25 and 50%, respectively

When NADH was added to concentration used for the enzymatic assay[8] to yeast alcohol dehydrogenase prior to incubation with iodoacetamide, no reduction of catalytic activity was detected Native and carboxymethylated enzyme filtered through a Sephadex G-100 column showed the same elution profile with identical elution volumes (51 ml) Such an elution volume corresponded to a molecular weight of 150 000 on the calibrated column

In order to interpret the effect of iodoacetamide on the NADH binding capacity of yeast alcohol dehydrogenase, it should be kept in mind (1) that iodoacetamide inactivation of this enzyme is due to the specific carboxymethylation of "essential" –SH groups[2] and (2) that the subunits of this protein are probably all identical and each of them contains only one "essential" –SH group[2] On the basis of these facts, if the –SH groups were necessary to bind NADH, a fully iodoacetamide inactivated yeast alcohol dehydrogenase should not bind any NADH, and a partially inactivated enzyme should bind NADH in amounts proportional to the residual activity On the contrary, our results show that fully inactivated enzyme retains almost full capacity for NADH binding as does 50% inactivated protein On the basis of these results we exclude a fundamental role of yeast alcohol dehydrogenase "essential" –SH groups in the binding of NADH to the enzyme protein The ability of NADH to protect this enzyme from iodoacetamide inactivation might be interpreted in two ways (a) the NADH binding sites might be very close to the "essential" –SH group or (b) the binding of NADH to the protein might effect a structural change in the enzyme such that the reactivity of –SH groups is impaired

The more powerful inhibition of the NADH binding capacity by maleimide and *N*-ethylmaleimide than by iodoacetamide could be due to the fact that the two former reagents react with –SH groups distinct from those termed "essentials"

The Sephadex G-100 filtration experiments seem to exclude a role of "essential" –SH groups in maintaining the quaternary structure in contrast to what is suggested by experiments of inactivation with PCMB[10] In conclusion, the present results show that in the case of yeast alcohol dehydrogenase, the "essential" –SH groups do not serve the function of binding the coenzyme

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