Cytosine deaminase: Structural modification studies

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Summary. Structural modification studies have shown that a cysteine, a histidine and possibly an arginine residue are involved in the catalytic process. The enzyme gave a single band on polyacrylamide gel electrophoresis, and the amino acid analysis showed it to contain a high proportion of hydrophobic residues, which was in agreement with the chemical modification results.

In 1952 Kream and Chargaff[4] described the hydrolytic deamination of cytosine and 5-methylcytosine by cytosine deaminase (cytosine aminohydrolase, EC 3.5.4.1) in a fraction precipitated with ammonium sulphate from crude extracts of baker's yeast. Ipata et al.[5] studied the enzymic properties of the partially purified enzyme (gel filtration on sephadex G100 and chromatography on DEAE-cellulose). We wish to report in this paper some chemical modification studies on this enzyme.

Materials and methods. Cytosine deaminase was purified from baker's yeast as described by Ipata et al.[5]. To obtain a sample which gave a single band on polycrylamide gel electrophoresis for the amino acid analysis and titration of the thiol groups, the gel filtration on sephadex G100 was carried out using a quarter of the column loading used by these authors; this resulted in better separation on this column and the subsequent DEAE-cellulose column. The enzyme was assayed as described by Ipata et al.[5].

The amino acid analyses were carried out in triplicate and ratios were calculated by the method of minimizing deviations from whole number ratios. Tryptophan was determined by a modification of the method of Barman and Koshland[6]. The following marker proteins were employed for the dodecyl sulphate-polyacrylamide gel electrophoresis: serum albumin, ovalbumin, lysoyme and β-lactoglobulin. Enzyme concentrations were measured using the optical density at 280 nm.

Results and discussion. Cytosine deaminase was found to have a mol.wt of approximately 32,000 using dodecyl sulphate-polyacrylamide gel electrophoresis as described by Weber and Osborn[7], while amino acid analysis gave a value of approximately 33,000; Ipata et al.[5] found 34,000 using gel filtration. The K_m for cytosine was found to be 2.0 mM.

The inactivation of the enzyme by mercury compounds (table 1) suggests that there is a sulphydryl group at the active site. The lack of reactivity of this group towards alkylating agents (e.g. iodacetate) could be due to one or both of the following reasons:

a) The active site is very confined. It is interesting to note that, of the mercury compounds studied, the smallest (mercuric acetate) is the most powerful inhibitor.

b) The active site is in a hydrophobic region of the enzyme, making the entry of ionic species particularly difficult; the amino acid analysis (table 2) shows the presence of a larger than usual number of hydrophobic residues. This has been shown to be the case with the related enzyme adenosine deaminase[8]; this effect has also been observed

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Table 1. Modification reactions carried out

Reagent	Concentration	pH	Inhibition (%)	Incubation Temperature (°C)	Time (h)
Mercuric acetate	10^{-5} M	7.0	26.2	4	0.25
Mercuric acetate	10^{-4} M	7.0	100	4	0.25
Mercuric acetate	5×10^{-5} M	7.0	100	4	0.25
Phenylmercuric acetate	10^{-4} M	7.0	49.5	4	0.25
p-Chloromercuribenzoate	10^{-3} M	7.0	90	RT	0.5*
N-Ethylmaleimide	10^{-3} M	6.2	0	4	0.25*
N-Ethylmaleimide	10^{-3} M	7.2	0	4	0.25*
Iodocetamide	2×10^{-3} M	8.0	0	RT	0.5*
Iodacetate	2×10^{-2} M	6.2	0	RT (dark)	24.0*
5.5-Dithio-bis-(2-nitrobenzoic acid)	5×10^{-5} M	7.0	0	RT	0.1*
Tetrathronate	3.32×10^{-2} M	7.0	0	4	0.25
o-Iodobenzoate	10^{-3} M	7.0	0	RT (dark)	0.5*
N-Acetylimidazole	64×10^{-3} M	7.2	0	RT	1.0*
Potassium cyanate	0.2 M	7.0	66	30	1.0*
Potassium cyanate	0.2 M	8.5	18	30	1.0*
Glyoxal	2×10^{-2} M	8.0	41	RT	19.0*
Phenylglyoxal	2×10^{-4} M	8.0	0	RT	20.0*

For reviews see Cohen[11], Vallee et al.[14] and Glazer[15]. RT, room temperature; * dialyzed for 24 h at 4 °C against tris buffer (0.03 M, pH 7.0).

Table 2. Amino acid analysis of cytosine deaminase

	n		n
Asp	37	Met	1
Thr	19	Ileu	15
Ser	22	Leu	19
Glu	27	Tyr	5
Pro	10	Phe	13
Gly	36	Lys	22
Ala	28	His	7
Cys	1	Arg	13
Val	31	NH_3	8
		Trp	(2)*

* Determined by the method of Barman and Koshland[6].

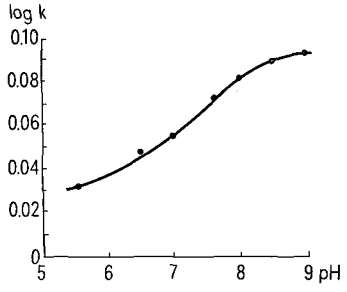

Photo-oxidation with methylene blue. Plot of log k against pH.

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with beef lung lactate dehydrogenase, where the amino acid analysis of a portion of the enzyme showed the cysteine residue concerned to be in a very hydrophobic region[9].

Substrate protection experiments, using cytosine as substrate and mercuric acetate, showed that the cysteine residue was at the active site (11.9% inhibition in the presence of the substrate, compared with 54.1% in the control). Titration of enzyme solution with p-chloromercuribenzoate as described by Ronca et al.[8] showed only one reactive –SH group present in the enzyme; the amino acid analysis showed only one cysteine residue present in the enzyme. The reaction of cyanate with this enzyme was probably with a thiol group. The inhibition was much less at pH 8.5 than at pH 7.0, which is in agreement with the observations of Stark[10] who studied the reaction of cysteine with cyanate; only carbamyl derivatives of amines are stable at both pH 7.0 and 8.5[11].

In photo-oxidation studies, 70% inhibition was observed with methylene blue (1×10^{-1} mg/ml) in 30 min, while 39% inhibition was observed with rose bengal (1.6×10^{-3} mg/ml). It is known that rose bengal is much more specific for histidine than methylene blue[12]. It seems likely that there is a histidine residue at the active site; again there is no reaction with alkylating agents (table 1). From data on the photo-oxidation of the enzyme by methylene blue at various pHs, the log of the rate of inactivation was plotted against pH[13].. The plot (figure) suggested that a residue with an ionizing group which has a pH of 7.1 is involved in the catalytic process; this would suggest histidine. From a series of experiments using phenylmercuric acetate, and photo-oxidation using methylene blue, it was concluded that 2 different groups were being modified by these reagents.

The inactivation of the enzyme by glyoxal may indicate that an arginine residue plays an important role. Interestingly, the enzyme is not inactivated by phenylglyoxal, indicating that the size of the reagent is probably important. These studies show that there is a cysteine, a histidine and possibly an arginine residue at the active site. This enzyme is the subject of further studies at these laboratoires.

A highly active glutathione peroxidase in red blood cells of normal and acatalasemic mice[1]

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Summary. The activities of catalase (E.C.1.11.1.6) and glutathione peroxidase (E.C.1.11.1.9) were compared in red blood cells from humans, ducks and normal and acatalasemic mice. In the cells from both strains of mice, an equally high activity of GSH-Px was found which could be inhibited completely by iodoacetate but was not sensitive to N-ethylmaleimide.

In comparative studies on catalase activities in red blood cells from various species, the duck (no activity) and the primates (high activity) are considered the extremes[3], whereas these red cells show a reversed distribution of glutathione peroxidase (GSH-Px). By selection and crossing of mouse mutants, Feinstein[4] was able to obtain red cells with normal or very low catalase activity within the same species. The question arose whether the red cells from the acatalasemic mice developed an adaptation in H_2O_2 removal by an increased GSH-Px activity.

Materials and methods. Human blood was provided by the hospital blood bank. Ordinary farm ducks were bled from the wing vein. The normal (Cs^a) and acatalasemic (Cs^b)

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