# SUBUNIT STRUCTURE OF GLYOXALASE I FROM YEAST

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## 1. Introduction

The glyoxalase system [1,2] catalyzes the conversion of 2-oxoaldehydes to the corresponding 2-hydroxyacids. The biological role of this enzyme system remains unsettled since its discovery in 1913, but a function in the detoxification of the strongly electrophilic 2-oxoaldehydes has been discussed. In particular the detoxification of methylglyoxal, which may be formed from dihydroxyacetone phosphate by enterobacteria in the alimentary canal, has been suggested as a role in mammals [3] . Racker resolved the system in yeast and found that one enzyme, glyoxalase I, catalyzes the formation of *S*-2-hydroxy-acylglutathione and a second enzyme, glyoxalase II, effects the hydrolysis of this thiolester [4] . Glyoxalase I (EC 4.4.1.5) has been studied most extensively of the two enzymes, and especially its catalytic properties have been analyzed (cf. [5] ). The steady-state kinetics of glyoxalase I are complicated by the spontaneous formation of a hemimercaptal adduct of glutathione (GSH) and the 2-oxoaldehyde; the enzyme acts on a mixture composed of GSH, 2-oxoaldehyde, and their corresponding hemimercaptal adduct. In our laboratory it was discovered that the kinetics were complex and included the effect of at least two components, e.g., GSH and the adduct [6–8] , and, if adduct was considered as substrate and GSH as inhibitor, the rate equation was non-Michaelian with respect to adduct and showed a non-linear inhibition by GSH [9–11] . The simplest explanation of these results was considered to be a branching reaction scheme composed of a one-substrate pathway with adduct as substrate and a two-substrate pathway involving GSH and 2-oxoaldehyde as substrates [9–11] .

The discovery that mammalian glyoxalase I from several sources was a molecule composed of two subunits [3,12–14] provided an alternative interpretation of the non-linearities observed in the analysis of the steady-state kinetics, namely cooperativity between binding sites localized on different subunits. The enzyme from yeast has a considerably lower molecular weight than the enzyme from mammalian sources [10] and it was therefore possible that the yeast enzyme was composed of only one subunit. If so, the non-linear kinetics of the yeast enzyme [10,11] cannot be explained by subunit interactions. Such a conclusion should also have implication for the interpretation of the non-linearities found with the mammalian enzymes. In the present paper the results of a study of the subunit structure of glyoxalase I from yeast are reported.

## 2. Materials and methods

Suppliers of chemicals and chromatographic media have been reported [3,14] . Glyoxalase I from yeast was obtained from Boehringer Mannheim. The assay of glyoxalase I activity was that described for the rat liver enzyme [14] . Protein concentration was calculated from $A_{260}$ and $A_{280}$ values [15] . Estimations of molecular weight by gel filtration and sodium dodecyl-sulfate–polyacrylamide gel electrophoresis were performed as described [3,14] . Amino acid analyses were carried out by Dr D. Eaker, Department of Biochemistry, Biomedical Center, University of Uppsala.

Peptide mapping of a tryptic digest of purified glyoxalase I was carried out by two-dimensional electrophoresis/chromatography as described [16] . The enzyme was first carboxymethylated by a procedure [17] slightly modified from [18] .

Lyophilized enzyme (about 2 mg) was dissolved in 0.5 ml 8 M urea/0.5 M Tris—HCl (pH 8.0) containing 2 mM EDTA and incubated at 37°C for 1 h with 10 mM dithiothreitol. Neutralized iodoacetic acid was then added to a concentration of 25 mM. After 1 h at 22°C the solution was passed through a column of Sephadex G-25 (1.5 × 10 cm) equilibrated with 0.5% NH₄HCO₃ and the protein was recovered by lyophilization. The protein was dissolved in 0.5 ml 0.5% NH₄HCO₃ and digested with L-(1-tosylamido-2-phenylethyl)-ethyl chloromethyl ketone-treated trypsin (1:100, by wt) for 5 h at 37°C and then lyophilized. The sample was dissolved in 0.1 M ammonia and applied to a Whatman 3 MM paper. Electrophoresis (at pH 6.5) was performed for 45 min (60 V/cm). The paper strip with the peptides was sewn onto another sheet of paper and the peptides were separated by descending chromatography for 16 h in the system n-butanol/acetic acid/water/pyridine (30/6/24/32, by vol.). Peptides were stained with a ninhydrin reagent.

## 3. Purification of glyoxalase I from yeast

A commercial preparation from Boehringer, Mannheim, of glyoxalase I (spec. act. 200 units/mg) was further purified.

### 3.1. Step 1: Isoelectric focusing

Isoelectric focusing of commercial enzyme (25 mg protein) was performed in a sucrose density gradient at 4°C in a 110 ml column (model 8101 from LKB Produkter). Ampholytes (1% w/w) were used to create a pH gradient in the range of pH 5–8. After focusing the contents of the column were collected in 1 ml fractions.

### 3.2. Step 2: Affinity chromatography

The pooled material (33 ml) from step 1 was applied to a column (1 × 25 cm) containing an adsorbent obtained by coupling of S-hexylglutathione to epoxy-activated Sepharose 6B (cf. [3]). The column, which was equilibrated with 10 mM Tris/HCl (pH 7.8) was washed with the same buffer supplemented with 0.2 M NaCl after application of the enzyme. The enzyme was then eluted with 3 mM S-hexylglutathione and 5 mM GSH dissolved in 10 mM Tris/HCl (pH 7.8).

### 3.3. Step 3: Chromatography on Sephadex G-100

The pooled material (9 ml) from step 2 was chromatographed on a Sephadex G-100 (Fine) column (4 × 43 cm), equilibrated with 10 mM Tris/HCl (pH 7.8).

The overall yield of enzyme was 43% and the specific activity rose to 1080 units/mg, corresponding to a 5.4-fold purification.

## 4. Results and discussion

Purified glyoxalase I from yeast had est. mol. wt 32 000 by gel filtration on Sephadex G-100, in agreement with [10]. Glyoxalase I from mammalian tissues appears in all known cases to have approx. mol. wt 50 000 [3,12–14]. It was considered that the difference in molecular weights might be an artifact resulting from partial digestion of the yeast enzyme after lysis of the yeast cells. Therefore, the molecular weight was also determined on a fresh yeast extract obtained by rupture (at 20 000 psi) of 10 g cells (bakers yeast), suspended in 30 ml 10 mM Tris/HCl (pH 7.8), in a Sorvall Ribi Cell Fractionator followed by centrifugation for 20 min at 19 200 × g. However, the estimate of the molecular weight was the same as in the case of the purified enzyme, indicating that the native enzyme has approx. mol. wt 32 000.

The remaining question was whether or not the yeast enzyme was composed of subunits like glyoxalase I from mammalian sources, [3,12–14]. Gel filtration on Sepharose CL-6B equilibrated with 6 M guanidium chloride showed no evidence for any component of mol. wt < 32.000. Neither was any evidence for dissociation of the enzyme into subunits obtained by sodium dodecylsulfate—polyacrylamide gel electrophoresis under a variety of conditions. The enzyme was preincubated with up to 20% 2-mercaptoethanol and 4 M urea before electrophoresis, but in all cases the molecular weight was estimated to about 32 000 by comparison with reference proteins.

To further corroborate the conclusion that glyoxalase I from yeast consists of a single polypeptide chain it was decided to analyze peptide maps after tryptic digestion of the enzyme. The amino acid composition of the enzyme was determined (table 1) and it was found that about 30 residues of arginine plus lysine were present per 32 000 dalton. Thus, about

Table 1
Amino acid composition of glyoxalase I from yeast

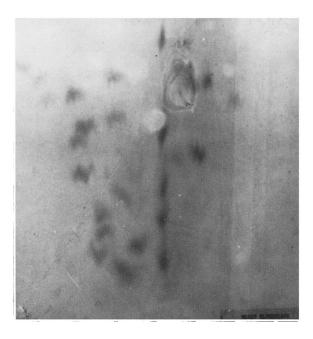| Amino acid | Residues/enzyme molecule (32 000 mol. wt) | |
| --- | --- | --- |
| | Found | Nearest integer |
| Asx | 30.4 | 30 |
| Thr | 14.1 | 14 |
| Ser | 20.1 | 20 |
| Glx | 29.8 | 30 |
| Pro | 14.7 | 15 |
| Gly | 25.6 | 26 |
| Ala | 10.2 | 10 |
| Cys | 14.5 | 15 |
| Val | 11.7 | 12 |
| Met | 2.0 | 2 |
| Ile | 13.6 | 14 |
| Leu | 20.1 | 20 |
| Tyr | 10.0 | 10 |
| Phe | 13.2 | 13 |
| His | 9.2 | 9 |
| Lys | 21.0 | 21 |
| Arg | 9.6 | 10 |



Fig.1. Tryptic-peptide map of glyoxalase I from yeast. Electrophoresis (horizontal direction: cathode, left; anode, right) was carried out at pH 6.5. Descending chromatography (vertical direction) was performed in n-butanol/acetic acid/water/pyridine (30/6/24/32, by vol.).

30 different peptides could be expected for a single polypeptide chain after tryptic digestion, whereas about 15 peptides would be obtained if the enzyme were composed of two identical subunits. The peptide map (fig.1) showed 27–30 tryptic peptides. Consequently, the enzyme cannot consist of two identical subunits and, in view of the failure to dissociate the protein into subunits under a variety of highly denaturing conditions, we therefore conclude that the enzyme molecule consists of a single polypeptide chain. This conclusion has far-reaching consequences for the interpretation of the non-hyperbolic kinetics of yeast glyoxalase I [10,11] in so far as it excludes the possibility of cooperative subunit interactions.

## Acknowledgements

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