THE AMINO ACID SEQUENCE AROUND THE REACTIVE CYSTEINYL RESIDUE OF BOVINE KIDNEY RHODANESE

F. BOSSA, C. CANNELLA, G. FEDERICI, D. BARRA and L. PECCI
Istituto di Chimica Biologica, Università di Roma-00185, Roma, Italy

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1. Introduction

Rhodanese (thiosulfate: cyanide sulfurtransferase, EC 2.8.1.1) is an enzyme that catalyses the transfer of the outer sulphur atom of thiosulfate to cyanide to form sulfite and thiocyanate. This enzyme has been obtained in very large amounts from bovine liver [1] and with a poor yield from bovine kidney [2]. The enzymes from the two sources appeared to be very similar both from the functional and the structural point of view [3], although the properties of the liver enzyme have been more thoroughly investigated [4–5]. For the liver enzyme Wang and Volini [6] obtained evidence that one of the cysteinyl residues is essential for enzymic activity. DeToma and Westley [7] described the selective alkylation of this residue with [^{14}C]iodoacetate, the purification of the labeled tryptic peptide and its amino acid composition.

We have recently described [8] a new procedure for obtaining in fairly good amounts the bovine kidney rhodanese. In order to establish the possible identity between this enzyme and that from liver, in the present paper we report the complete amino acid sequence of the labeled cysteinyl peptide obtained after kidney rhodanese according to the same procedure employed for the liver enzyme.

2. Experimental

Crystalline bovine kidney rhodanese was prepared as previously described [8]. A sample of 150 mg of two times crystallised enzyme was subjected to the carboxymethylation procedure described by DeToma and Westley [7] for the liver rhodanese, using [^{14}C]-iodoacetic acid (25 mCi/mmole, Radiochemical Centre, Amersham). Time course of the reaction was followed by measuring the enzymatic activity according to the method of Sorbo [9]; complete inactivation was achieved after 45 min. Radioactivity assay performed after extensive dialysis against water plus 1 mM mercaptoethanol showed that one mole of iodoacetic acid was incorporated per mole of inactive monomer. Carboxymethylated rhodanese was digested with trypsin (Worthington, TPCK treated) according to the procedure employed for the liver enzyme [7]. Tryptic peptides were analyzed by fingerprinting technique on cellulose thin layer plates as previously reported [10]. After staining with ninhydrin, the zones corresponding to the various spots were scraped off and counted for radioactivity in a Packard Tri-Carb Model 3380 liquid scintillation spectrometer equipped with the Model 544 absolute activity analyzer. Fractionation of tryptic peptides was performed on a Sephadex G-25 column (4 × 130 cm) eluted with 5% acetic acid. Effluent was monitored for absorbance at 280 nm and radioactivity. The radioactive peptide was further purified by paper chromatography using n-butanol: pyridine: acetic acid:water (15:10:3:12) as solvent. A guide strip cut from the chromatogram was scanned for radioactivity with a Model 7201 Packard scanner and then stained with ninhydrin. The corresponding radioactive zone was eluted with 10% acetic acid.

Amino acid compositions of peptides (about 25 nmoles) were determined with a BioCal BC 200 instrument (single column system) after hydrolysis with 6 N HCl at 110° for 24 hr.

The sequence of the peptides was established with the dansyl-Edman technique [11]. Dansyl-amino acids were identified on polyamide plates [12] developed with the solvent system described by Hartley [13].

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An aliquot of the radioactive peptide (0.8 mnmoles) was digested with thermolysin in 0.05 M ammonium bicarbonate and 1 mM $CaCl_2$ for 2 h at 37°. The digest was subjected to high voltage electrophoresis in pH 1.9 buffer (formic acid : acetic acid : water, 2 : 8 : 90) in a Varso cooled tank (50 V/cm for 50 min).

Digestion of peptides with carboxypeptidases A and B (Worthington, DFP-treated) was performed essentially according to Ambler [14]. The release of amino acids was followed by applying suitable, acidified aliquots of the digestion mixture on the amino acid analyzer.

3. Results

The peptide map of 0.1 mg of labeled rhodanese shows the presence of a single, well resolved, radioactive peptide (fig. 1). The elution pattern of the tryptic peptides from Sephadex G-25 is reported in fig. 2. The fraction corresponding to the radioactive peak was further purified by paper chromatography, as described under Experimental.

The amino acid composition of the labeled peptide (K-3) is given in table 1, where the amino acid composition of the corresponding peptide obtained by DeToma and Westley from liver rhodanese is also reported for comparison (L). The N-terminal amino acid of K-3 was found to be lysine by dansylation. Sequencing of

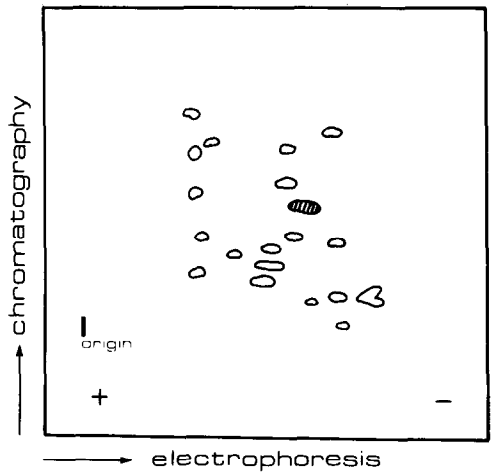

Fig. 1. Peptide map of the tryptic digest (0.1 mg) of carboxymethylated rhodanese. Electrophoresis at pH 2.0 was followed by chromatography in pyridine–n-butanol–water–acetic acid (12 : 15 : 8 : 3, by vol). The shaded area represents the radioactive peptide.

0.5 μmoles of K-3 by dansyl-Edman procedure led to the partial sequence Lys–Val–Asx–Leu–Thr–(Lys, Pro)–Leu–Ile–Ala–(Thr)–CMCys–Arg. In order to solve the problem of the uncertain assignment of residues 6, 7 and 11 and the question whether the third residue exists as aspartic acid or asparagine, K-3 was digested with thermolysin. The amino acid composition of the three main thermolytic fragments (TL-1, TL-2 and TL-3) is reported in table 1. TL-1 was sub-

Table 1
Amino acid composition of the carboxymethylated peptides from bovine liver and kidney rhodaneses.

Amino Acid	K–3	$L_{(7)}$	TL 1	TL 2	TL 3
CM-Cys	0.62 (1)	0.95 (1)			0.61 (1)
Asp	1.00 (1)	1.91 (2)	1.13 (1)		
Thr	1.62 (2)			0.94 (1)	0.92 (1)
Ser		0.98 (1)			
Glu		2.10 (2)			
Pro	0.86 (1)			0.82 (1)	
Gly		1.06 (1)			
Ala	0.95 (1)	0.90 (1)			1.05 (1)
Val	1.00 (1)	0.95 (1)	0.87 (1)		
Ile	0.95 (1)	0.91 (1)			0.89 (1)
Leu	1.95 (2)			1.00 (1)	0.82 (1)
Phe		2.00 (2)			
His		0.88 (1)			
Lys	1.72 (2)	0.96 (1)	1.00 (1)	1.00 (1)	
Arg	1.00 (1)	0.88 (1)			1.00 (1)

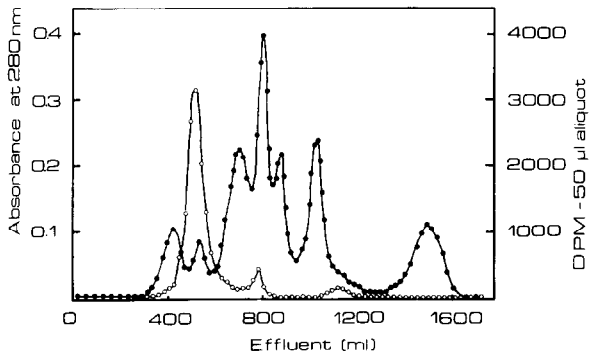

Fig. 2. Gel filtration on Sephadex G-25 of the tryptic peptides of labeled rhodanese: (●–●–●) absorbance; (○–○–○) radioactivity.

jected to two cycles of the Edman degradation and then applied on the amino acid analyzer; by this means the third residue was shown to be aspartic acid.

Sequencing of TL-2 and TL-3 gave: TL-2, Leu–Thr–Lys–Pro; TL-3, Leu–Ile–Ala–Thr–CMCys–Arg. A suitable amount of TL-3 was also digested with carboxypeptidases. Carboxypeptidase B released only arginine, while inculation for 5 hr with both carboxypeptidase A and B released arginine, carboxymethylcysteine, threonine and alanine in equimolar amounts.

The complete sequence of the carboxymethylated peptide must be: Lys–Val–Asp–Leu–Thr–Lys–Pro–Leu–Ile–Ala–Thr–CMCys–Arg.

4. Discussion

Our data for the bovine kidney rhodanese confirm those obtained by DeToma and Westley [7] for the corresponding liver enzyme. In both cases one sulphydryl group per monomer is rapidly carboxymethylated in the native enzyme and this reaction is accompanied by a complete loss of catalytic activity. We have already suggested, on the basis of a fluorescence study [15], the formation of a persulfide group at the active site of rhodanese when the sulfane sulfur of thiosulfate is bound to the enzyme. This highly reactive cysteinyl residue is the obvious candidate for being the site where persulfide is formed.

Furthermore, the existence of the persulfide group explains the requirement, observed both for the liver and the kidney rhodanese, of the treatment with cyanide in order to obtain a stable carboxymethylation of the enzyme.

Despite the strict similarities between the functional properties of the liver and kidney enzymes, amino acid composition of the labeled peptide obtained from kidney rhodanese is markedly different from that obtained in identical conditions by DeToma and Westley [7] for the liver enzyme.

It should however be pointed out that valine is the N-terminal residue of the labeled peptide from the liver enzyme and that the same could occur for the peptide of the kidney rhodanese with a different, but theoretically possible, tryptic cleavage.

Unfortunately a more detailed comparison between the two peptides is not possible since the amino acid sequence of the liver enzyme peptide is not available so far. Such comparison would clarify if the differences found in the amino acid composition of the corresponding fragments obtained from two functionally identical enzymes, synthesized in different organs of the same animal, must be considered, at least in part, originated from conservative substitutions.

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