RAT LIVER ALCOHOL DEHYDROGENASE. CHARACTERISATION OF ALKYLATED CYSTEINE RESIDUES IN THE CARBOXYMETHYLATED PROTEIN

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1. Introduction

Alcohol dehydrogenase (EC 1.1.1.1) from rat liver may be obtained in multiple forms and the electrophoretic pattern is influenced by the pretreatment, e.g. by thiols [1]. The explanation of the multiple forms is unknown but the effect of thiols may at least in some of these cases suggest a role of cysteine residues. These have not been fully characterized in the enzyme but one cysteine difference is known between the rat and horse proteins [2]. The determination of the number of cysteine (or half-cystine) residues in rat liver alcohol dehydrogenase is therefore of special interest in the structural study of the enzyme and is reported in the present work. 16 Unique carboxymethylcysteine (CM-cysteine) residues were found in the polypeptide chain of the reduced and [^{14}C] carboxymethylated protein and the amino acid sequences around them are given.

2. Materials and methods

Rat liver alcohol dehydrogenase was prepared with ethanol as stabilizing agent [1]. The pure enzyme was reduced and carboxymethylated [2] with iodo [2-^{14}C] acetate. This protein derivative was digested with TLCK-treated [3] chymotrypsin (1:100, by weight) in 0.1 M NH_4HCO_3 for 4 hr at 37°. After fractionation of the digest on Sephadex G-50 radioactive peptides were purified by paper electrophoresis and chromatography and analyzed for composition, end-groups and amino acid sequences, as previously described [2].

3. Results

A chymotryptic digest of [^{14}C] carboxymethylated rat liver alcohol dehydrogenase (100 mg with 5×10^7 cpm) was separated into six fractions [4] by chromatography in 0.1 M NH_4HCO_3 on Sephadex G-50 (column dimensions: 2.5 × 100 cm; recovery of radioactivity: over 95%). Purification of ^{14}C-peptides in each fraction yielded a total of 12 different major radioactive peptides. Ten of these were pure and their data are given in table 1. The remaining two peptides (CS1:1 and CS2:4) were not completely pure and no N-terminal amino acids could be attributed to them. Redigestion with trypsin, however, produced one radioactive fragment from each of these two peptides. The two fragments were obtained pure and they are also included in table 1 (CS1:1T3 and CS2:4T3). All radioactivity found in major peptides in a chymotryptic digest of [^{14}C] carboxymethylated rat liver alcohol dehydrogenase is thus represented in table 1.

The amino acid sequences of the radioactive peptides were established by the dansyl-Edman method and are given in table 2. The structures of five of the larger peptides were not obtained by complete degradations of the original peptides, but by additional analysis of fragments produced by redigestion with proteolytic enzymes, as indicated in table 2. These fragments were also obtained from other digests and

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Table 1

Data for radioactive peptides from [^{14}C] carboxymethylated rat liver alcohol dehydrogenase.

Property	Peptide											
	CS1:1T3	CS2:3	CS2:4T3	CS3:1	CS3:4	CS3:7	CS3:9	CS3:10	CS4:2	CS4:3	CS5:1	CS5:11
No. of purification steps	5	3	4	3	4	5	5	5	4	4	3	4
Recovery (%)	9	12	25	20	19	26	6	5	27	10	19	5
Electrophoretic mobility [5] at pH 6.5	0	+0.20	0	+0.69	+0.20	0	0	0	+0.37	+0.31	+0.41	−0.62
Composition												
Cys(Cm)	0.9 (1)	1.8 (2)	0.8 (1)	0.8 (1)	0.7 (1)	0.8 (1)	0.8 (1)	2.5 (3)	0.7 (1)	1.7 (2)	1.0 (1)	0.1 (1)
Asp	– –	– –	– –	3.0 (3)	2.0 (2)	– –	1.0 (1)	1.0 (1)	1.1 (1)	1.2 (1)	– –	– –
Thr	– –	– –	– –	1.0 (1)	0.9 (1)	1.8 (2)	– –	– –	– –	1.2 (1)	– –	– –
Ser	1.1 (1)	4.2 (4)	– –	– –	2.3 (2)	1.3 (1)	– –	1.1 (1)	– –	– –	– –	1.1 (1)
Glu	– –	1.2 (1)	– –	1.1 (1)	– –	– –	– –	1.3 (1)	– –	1.1 (1)	– –	– –
Pro	– –	1.8 (2)	– –	1.0 (1)	– –	0.9 (1)	1.9 (2)	0.7 (1)	– –	– –	– –	1.3 (1)
Gly	1.1 (1)	2.3 (2)	– –	1.0 (1)	1.0 (1)	1.1 (1)	1.1 (1)	1.2 (1)	– –	– –	1.8 (2)	1.4 (1)
Ala	– –	2.2 (2)	– –	1.0 (1)	1.2 (1)	2.0 (2)	– –	– –	– –	– –	– –	– –
Val	1.2 (2)*	4.0 (5)*	– –	– –	1.0 (1)	2.6 (3)	1.5 (2)*	– –	1.1 (1)	– –	– –	– –
Ile	0.8 (1)	0.6 (1)*	– –	1.0 (1)	– –	– –	0.5 (1)*	0.6 (1)	– –	– –	1.1 (1)	0.8 (1)
Leu	– –	0.6 (1)	– –	– –	– –	– –	0.9 (1)	0.8 (1)	1.0 (1)	1.0 (1)	– –	– –
Tyr	– –	– –	– –	1.0 (1)	– –	– –	– –	– –	– –	– –	– –	– –
Phe	– –	– –	– –	– –	– –	0.8 (1)	– –	– –	– –	– –	0.8 (1)	1.0 (1)
Lys	1.0 (1)	– –	1.0 (1)	– –	– –	1.0 (1)	2.1 (2)	2.3 (2)	1.0 (1)	0.8 (1)	– –	1.0 (1)
His	– –	1.1 (1)	– –	– –	1.0 (1)	– –	– –	0.7 (1)	– –	– –	– –	1.5 (2)
Arg	– –	– –	– –	– –	0.8 (1)	– –	– –	0.8 (1)	– –	– –	– –	0.9 (1)
Total	7	21	2	11	11	13	11	14	5	7	5	10
N-terminus	Ser	Ser	Cys(Cm)	Gly	Val	Val	Cys(Cm)	Cys(Cm)	Asp	Cys(Cm)	Ile	Ser

Peptides were obtained as described in the text. Compositions are given as molar ratios without correction for destruction or incomplete hydrolysis.

*Sequence analysis reveals that the peptides contain Val–Val (CS1:1T3), Val–Ile–Val (CS2:3) or Val–Ile (CS3:9); incomplete hydrolysis of these bonds explains the low recoveries of Val and Ile.

Table 2

Amino acid sequences of radioactive peptides from [^{14}C] carboxymethylated rat liver alcohol dehydrogenase.

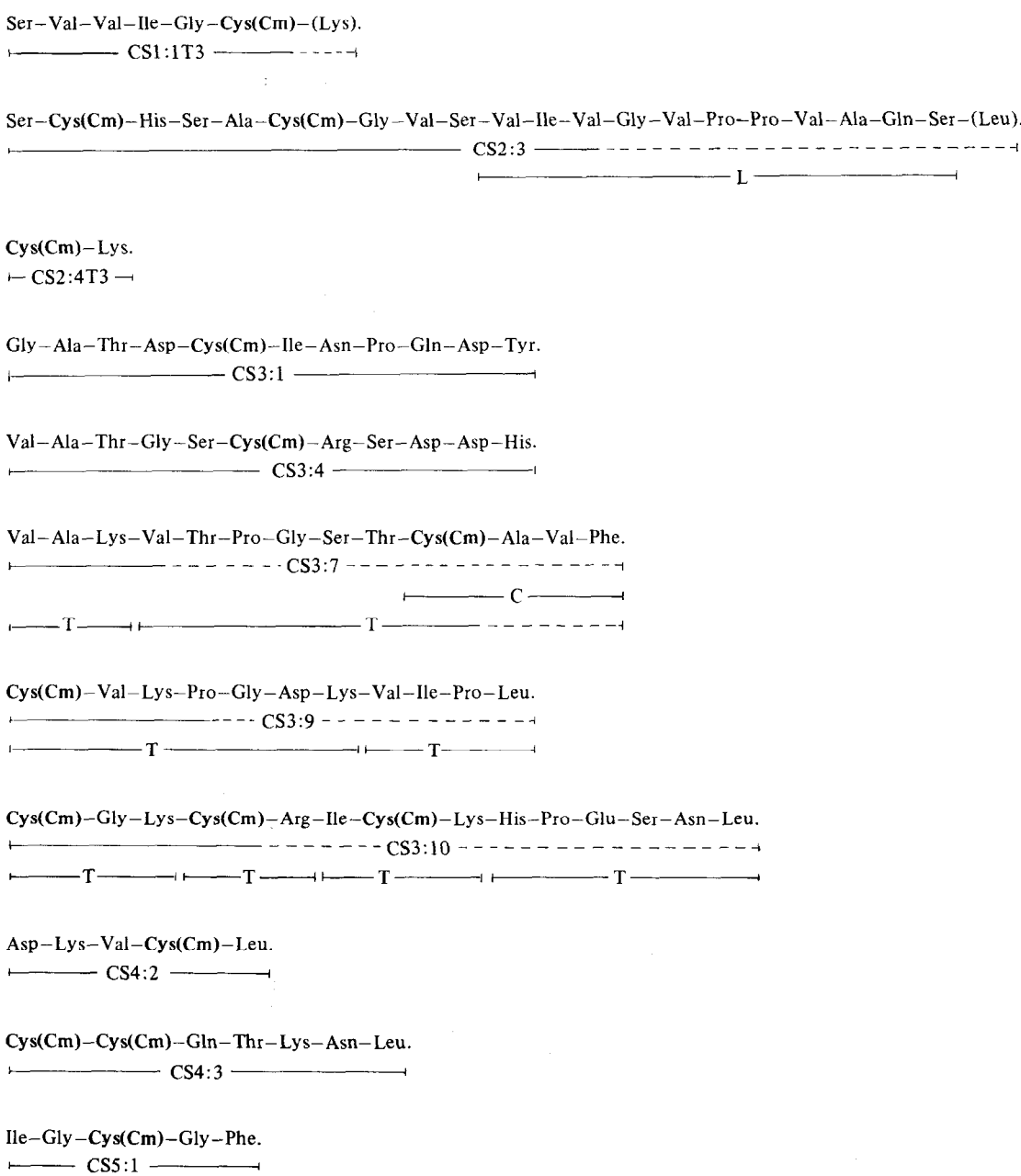

Table 2 (continued).

Ser–**Cys(Cm)**–Arg–Gly–Lys–Pro–Ile–His–His–Phe.
CS5:11
T T

Parts of peptides analysed by the dansyl-Edman method are indicated by solid lines, remaining parts by broken lines. Peptides obtained by redigestion with thermolysin, trypsin or chymotrypsin are indicated by L, T and C, respectively.

further work revealed why no N-terminal amino acids were found in the two peptides above: CS1:1 has N-terminal serine (from TS1:1T3) which was not detected due to the size of the peptide and the lability of dansyl-serine. CS2:4 is originating from the N-terminus of the whole protein chain and therefore blocked by an acetyl group [2].

Two minor peptides, present in small amounts in the chymotryptic digest and produced by cleavages at less sensitive bonds, could also be identified: Cys(Cm)–Gly–Lys (from the N-terminal part of CS3:10; due to a split at the Lys–Cys(Cm) bond, cf. table 2) and Cys(Cm)–Ala–Val–Phe (from the C-terminal part of CS3:7; due to a split at the Thr–Cys(Cm) bond, cf. table 2).

4. Discussion

16 Unique CM-cysteine residues were found by sequence analysis of the major radioactive peptides (table 2) in the chymotryptic digest of [^{14}C] carboxymethylated rat liver alcohol dehydrogenase. It is unlikely that minor peptides, of which two were identified, could account for additional unique CM-cysteine residues. The protein chain of rat liver alcohol dehydrogenase is therefore concluded to contain 16 cysteine (or half-cystine) residues. The characterisation of these residues is of value in studies of the multiple forms of the protein [6].

The positions of the CM-cysteine residues in the peptides of rat liver alcohol dehydrogenase and of the cysteine residues in the structure of the homologous [2] horse enzyme [7] may be compared. It is then revealed that 13 of these residues occur at equivalent positions in the two proteins, three are peculiar to the rat protein (the CM-cysteine in peptide CS3:9, the first in CS4:3 and the second in CS2:3) and one to the horse protein (at a possition corresponding to the first serine in CS2:3). It is thus clear that several differences involving cysteine residues exist between the two enzymes and that rat liver alcohol dehydrogenase contains even more cysteine (half-cystine) residues than the horse protein.

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References

[1] O. Markovič, H. Theorell and S. Rao, Acta Chem. Scand. 25 (1971) 195
[2] H. Jörnvall and O. Markovič, European J. Biochem. 29 29 (1972) 167.
[3] E. Shaw, M. Mares-Guia and W. Cohen, Biochemistry 4 (1965) 2219.
[4] H. Jörnvall, European J. Biochem. 14 (1970) 521.
[5] R.E. Offord, Nature 211 (1966) 591.
[6] H. Jörnvall, to be published.
[7] H. Jörnvall, European J. Biochem. 16 (1970) 25.