THE INTER-CHAIN DISULFIDE BONDS OF A μ-CHAIN DISEASE PROTEIN

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Received 23 August 1974

1. Introduction

Heavy chain disease proteins are homogeneous Ig molecules lacking the light polypeptide chains and composed of incomplete heavy chains, present in the serum of patients with lymphoproliferative diseases. While 30 cases of γ-chain disease and approximately 60 cases of α-chain disease have been recorded to date, only 6 cases of μ-chain disease have been reported and very few of these proteins have been characterized in detail. Recently two new μ-chain disease (μCD) proteins occurring in the serum of two patients of Ivory Coast origin were reported from our laboratory [1] and one of them (μCD protein BO) could be studied more fully. We have undertaken studies of the primary structure of the μCD protein BO and we present here the first data concerning the number of the interchain disulfide bonds and the amino acid sequence of the peptides containing the cysteinyl residues involved in these –S–S– bridges.

2. Materials and methods

The isolation of μCD protein BO and of the monoclonal K type IgM DU from the patients sera is fully described elsewhere [2]. Previous physicochemical and immunological characterization of protein BO showed that the native molecule had a sedimentation rate of s°_{20w} = 11.7 S and was constituted of 10 incomplete μ-chains interlinked by covalent bonds.

Abbreviations: CM-Cys SO_2: carboxymethyl cystein sulphone; Met SO_2: methionine sulphone.

The partial reduction and alkylation of the native proteins was performed following Miller and Metzger [3] with 10 mM D.T.T. and a 10% equivalent excess of ^{14}C iodoacetic acid which had specific activity of 7.5×10^8 cpm/mol. The reduced–alkylated (RA) polypeptide chains were isolated by gel-filtration on Sephadex G 100 in 1 M acetic acid.

Pepsin–trypsin digestion of the RA proteins and the purification of the radioactive peptides were performed according to Frangione and Milstein [4].

NH_2-terminal residues were determined by the Dansyl method [5] and the Dansyl derivatives were identified as described by Woods and Wang [6]. The serine/threonine and glutamic/aspartic acid derivatives were resolved with solvent III according to Crowshaw et al. [7].

COO-terminal amino acids were determined by H.V.E. at pH 2.1 of the carboxypeptidase A digests. The digestion was effected at an enzyme/substrate ratio 1/50 w/w in 0.1 M ammonium bicarbonate buffer pH 8.2 for 1/2 and 24 hr at 37°C.

The sequential amino acid degradation was effected by the Dansyl–Edman procedure [10].

The amino acid content of the proteins and peptides were performed using a Jeol 5 AH analyser. The proteins were hydrolysed in 6.7 N HCl in vacuum at 100°C for 16 hr, 24 hr and 48 hr and the peptides for 20 hr.

The ^{14}C radioactivity of the samples was measured in a Packard Tricarb 2425 scintillation system and corrected for quenching and efficiency.

The protein concentrations were obtained by O.D. determinations at 280 nm in 0.25 M acetic acid and the extinction coefficients $E^{1\%}_{280}$ were 13.5 for IgM DU and 10.0 for μCD protein BO.

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3. Results and discussion

The number of CM-Cys residues of μCD protein BO was measured in two ways: a) by radioactive counting of the molar specific activity associated to known amounts of the RA protein. The material employed was proved to be devoid of J-chain contaminants which could have interferred in the CM cysteinyl measurement. Values ranging from 2.8 to 3.1 CM-Cys residues were obtained in triplicate measurement per mol of polypeptide chain (mol. wt. = 5.5×10^4). Under the same experimental conditions it has been fully documented that all the interchain –S–S– bonds are specifically cleaved with the labelling of four Cys residues per μ-chain of 19 S IgM [3]. b) By amino acid analysis of the same RA material a value of 3.1 CM-Cys residues was obtained when the carbohydrate-free value of the molecular weight was taken into account (mol. wt. = 4.64×10^4).

The good agreement between the two methods strengthens the conclusion that three disulfide bridges are involved in the inter-chain linkage of the native protein BO.

In order to locate these Cys residues along the sequence of the μ-polypeptide chain the peptic–tryptic peptides were isolated and purified. After the oxidation step five labelled peptides were obtained and were numbered. P.T.-1; P.T.-2a; P.T.-2b; P.T.-3 and P.T.-4 according to their mobilities at pH 3.5 as shown in fig. 1. Their amino acid composition and sequence are shown in table 1 and table 2 respectively.

The comparison between the amino acid sequences of the Cys peptides from protein BO and the known sequences of the μ-polypeptide chain [9,10] shows the following:

1) Peptides P.T.-2b and P.T.-3 represent the C-terminal sequence of the μ-polypeptide chain. The Cys residue at position 570 (Gal μ-chain numbering) is involved in the second μ–μ-interchain (intra-subunit) bond from the N-terminal. The Met_{563}/Thr and Thr_{569}/Met interchanges are to be noted. The absence of the ultimate residue Tyr in peptide P.T.-2b could be explained by its partial cleavage due to serum or intracytoplasmic exopeptidase as reported for the C-terminal of the α-chains in IgA [11].

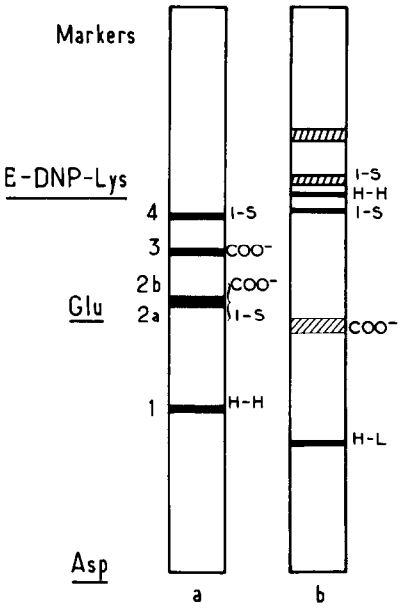

Fig. 1. Diagram showing the radioactive bands obtained by chemical typing at pH 3.5; a) μCD protein BO; b) μ-chain from Waldenström IgM DU. The anode is at bottom and the numbering of the peptides is from (+) to (–).

2) The peptides P.T.-2a and P.T.-4 are homologous with the sequence 405–410 (Gal numbering). The Cys-residue in position 408 has been demonstrated to participate in the μ–μ-inter-chain (inter-subunit) –S–S– bond. The residue $Glux_{400}$ found in protein BO corresponds to residue Asx_{400} in IgM Ou and Gal sequences [9,10] but it has been found in the inter-subunit Cys peptides isolated from IgM He [12].

3) Peptide P.T.-1 showed less homology to the corresponding one between residue 330 and 340 (Gal numbering) around the Cys_{342} involved in the first μ–μ-interchain (intra-subunit) disulfide bond from the N-terminal. To obtain maximal homology with this sequence a two residue gap had to be introduced in the sequence of peptide P.T.-1. With this ordering the interchanges Met_{331}/Gly; Val_{333}/Asp and Ile_{340}/Glu are apparent. The finding that the peptide P.T.-1 could also be isolated from the $(Fc\mu)_5 t^{60}$ fragment [13] is additional evidence that we are dealing with the peptide containing the Cys residue involved in the first μ–μ-interchain (intra-subunit) bond.

These results suggest that the $C\mu_3$ and $C\mu_4$ homology regions containing the two μ–μ-interchain

Table 1
Amino acid composition of a peptic–tryptic CM-Cys SO_2-peptide isolated from the RA μCD protein BO

Peptides	1	2a	2b	3	4
Lys					
His					
Arg					
CM-Cys SO_2	0.2	0.4	0.3		0.2
Asp	1.9		1.0	1.0	
Thr	0.9		1.8	1.9	
Ser	0.2		0.9	1.0	1.0
Glu	1.1	2.0			1.0
Pro	0.9				
Gly	1.0		1.3	1.0	
Ala	0.9		1.0	1.0	1.0
Val				0.8	
Met SO_2			0.8	0.9	
Ileu		1.0			
Leu					
Tyr				0.9	
Phe					
N-terminal	Ser	Ileu	Val	Val	Ala
Electrophoretic (b) mobility pH 6.5	1.0	0.92	0.60	0.51	0.73
Carbohydrates	–	–	–	–	–

(a) Expressed as moles of amino acid per mole of peptide.
(b) Expressed as fractions of the distance between ε-DNP-L-Lysine and Aspartic acid.

(intra-subunit) and the inter-subunit disulfide bond are present in the μCD protein BO while the sequence containing the Cys residue involved in the μ–L bond is absent. We are now studying the location and the extend of the deletion taking place in the N-terminal moiety of the protein BO. More intriguing are the residue changes and deletions observed in the μCD protein BO surrounding the interchain Cys residues, especially in the peptide P.T.-1. As the aminoacid sequences around the disulfide bridges are highly conserved in the immunoglobulin molecules one may wonder if, and to what extend aminoacid changes occurred in the rest of the polypeptide chains of protein BO. Whether this molecular variation is attributable to the neoplastic nature of the cells synthesising the protein or whether it represents a new subclass of human IgM is currently under investigation. Preliminary results would favour this latter hypothesis.

Acknowledgements

The competent technical assistance of Mr R. Miglierina for amino acid quantitative determination is acknowledged. This work was supported by Research Grant No. 73.4.033.2 from INSERM.

Table 2
Sequence of carboxymethyl cysteine peptides isolated from the reduced-alkylated μCD protein BO

Peptide	Function	Sequence	
		330 340	
	μ–μ	–Ser–Met–Cys–Val–Pro–Asp–Glx–Asx–Thr–Ala–Ile–Arg–	(a)
P.T.-1	(intra-subunit)	–Ser–Gly–Cys–Asp–Pro–Asp —— —— Thr–Ala–(Glu)	(b)
		405 410	
		Ala–Ser–Ileu–Cys–Glu–Asp	(a)
P.T.-2a	μ–μ	Ileu–Cys–Glux–Glux	
P.T.-4	(inter-subunit)	Ala–Ser–Ileu–Cys–Glux	
		562 570	
	μ–μ	Val–Met–Ser–Asx–Thr–Ala–Gly–Thr–Cys–Tyr	(a)
P.T.-2b	intra-subunit	Val–Thr–Ser–Asx–Thr–Ala–Gly–Met–Cys	
P.T.-3	(C-terminal)	Val–Thr–Ser–Asx–Thr–Ala–Gly–Met–Cys–Tyr	

(a) The sequence and numbering of reference Gal IgM. [10].
(b) (Glu) was assessed as C-terminal from the amino acid composition of P.T.-1.
——7 = Dansyl–Edman degradation step.
⟵ = Carboxypeptidase A degradation.

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