THE *N*-TERMINAL SEQUENCE OF A HUMAN γ1 CHAIN OF ALLOTYPE Gm(a⁻f⁺)

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1. Introduction

Immunoglobulin light chains, of both classes κ and λ, have been put into subgroups, based on the sequences of their variable halves [1–3]. In view of the similarity in sequence of the γ1 heavy chains Daw and Cor [4] and the μ chain Ou [5], in contrast to the sequence of the γ1 chain Eu [6], it seemed likely that there might be subgroups of heavy chain variable regions.

We have determined the sequence of the first 24 residues of a γ1 chain, Ste, Gm(a⁻f⁺) and find that 18 of the positions are occupied by the same residue as in Eu heavy chain. The similarity in sequence of the γ1 chains Ste and Eu is of the same order (75% of identical residues) as that of the γ1 chains Daw and Cor and μ chain Ou (70%). It is postulated, therefore, that Cor, Daw and Ou belong to one subgroup and Eu and Ste to another. These subgroups, however, are not correlated with the Gm groups (f⁺) and (z⁺), which are related to a single amino acid substitution in the constant region of the Fd-fragment [4,7], since the μ chain, Ou, does not express Gm groups and has a different constant region sequence from that of the γ chains.

2. Methods and materials

Immunoglobulin G (IgG) was isolated from the plasma of a patient (Ste) who was suffering from myelomatosis. The immunoglobulin was precipitated from the plasma with sodium sulphate (18% w/v), and purified by fractionation on a column of DEAE-Sephadex A-50 using gradient elution from 0.0175M- to 0.2M-sodium phosphate, pH 6.2. Heavy chains were prepared, digested with cyanogen bromide and totally reduced as described previously [8].

3. Results

The *N*-terminal amino acid of the heavy chain (Ste) was investigated by the dansyl technique, but no *N*-terminal residue could be detected. It is a common feature of heavy chains to have pyrrolid-2-one-5-carboxylic acid (PCA) as *N*-terminal residue.

The heavy chain was cleaved by cyanogen bromide and several large fragments and four small fragments were separated on a column of Sephadex G-100 in 6M-urea-0.2M-sodium formate, pH 3.3, after total reduction. One of the small fragments was the C-terminal octadecapeptide of the heavy chain and it was argued that the other three small fragments, namely 5 (50 residues), 6a (38 residues) and 6c (18 residues) must have come from the *N*-terminal part of the heavy chain, since the methionine residues in the constant region (commencing at residue 117) [4], occur at residues 252, 358 and 428 [7]. The *N*-terminal residues of fragments 5 and 6a were lysine and glutamic acid respectively. The *N*-terminal residue of 6c could not be detected; it was, therefore, probably the *N*-terminal fragment and was sequenced as shown in fig. 1. The *N*-terminal dipeptide isolated from 6c.Tl.Cl. was characterised by comparing its electrophoretic mobility at pH 6.5 with that of synthetic PCA-Val. The electrophoretic mobilities of the other peptides of 6c established that the two glutamic residues were

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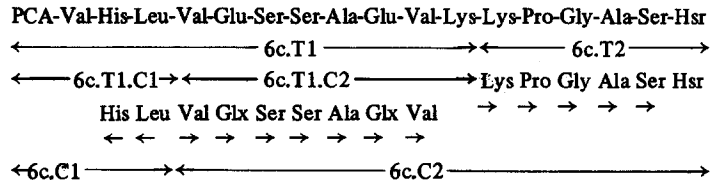

Fig. 1. Amino acid sequence of fragment 6c. Sequence determination from the *N*-terminal end by the dansyl-Edman technique [9] →, and from the *C*-terminal end by carboxypeptidase A digestion ←. 6c.T1 and T2 are tryptic peptides, 6c.C1 and C2 are chymotryptic peptides and 6c.T1.C1 and 6c.T1.C2 are chymotryptic peptides of T1.

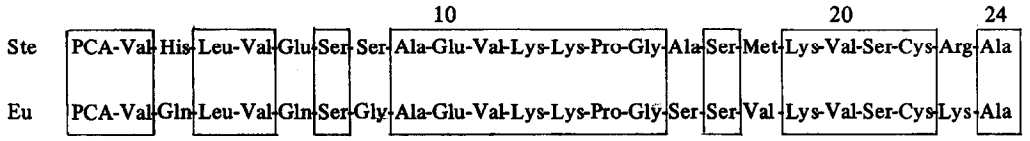

Fig. 2. Comparative sequence of human γ1 chains Ste and Eu [6].

present as glutamic acid. The dipeptide PCA-Val was also isolated from totally reduced whole heavy chain, by digestion with chymotrypsin and carboxypeptidase A, and fractionation on Dowex 50(H^+); thus confirming that fragment 6c was indeed *N*-terminal.

In all heavy and light chains so far sequenced, a cysteine residue occurs at about position 22. The *N*-terminal sequenee of fragment 5 is: Lys-Val-Ser-Cmc-Arg-Ala- and it is proposed that this fragment comes next to 6c, giving the *N*-terminal 24 residue sequence shown in fig. 2. The sequence is very similar to that of the first 24 residues of the γ1 chain Eu [6]. The *N*-terminal sequence of fragment 6a is Glx-Thr-Arg-Val-Thr-Ile-Thr-Ala-Asx, which is similar to the sequence of γ1 chain Eu from 65-73 (the identical residues are underlined). It seems most likely therefore, that the three cyanogen bromide fragments can be aligned in the order 6c-5-6a. Fragments 5 and 6a were linked by a disulphide bond in the partially reduced heavy chain, as would be expected from the arrangement suggested above.

4. Discussion

The similarity in sequence between the *N*-terminal 24 residues of Ste heavy chain reported here, with that of Eu heavy chain [6] is of the same order (75% identical residues) as the similarity between the variable regions of Daw and Cor heavy chains (70% identity) [4]. The sequence of the *N*-terminal 106 residues of a μ chain (Ou) has been reported [5], and this was also very similar to the sequence of Daw and Cor. Comparison of the variable region sequences of Eu and Daw shows that only 30% of the positions are occupied by the same residue.

Both κ and λ light chains have been divided into subgroups based on the extent of the variability found in their variable halves and in view of the very striking similarity in sequence of the variable regions of the heavy chains Daw, Cor and Ou, it is suggested that these represent a subgroup of heavy chain, and that Eu and Ste represent another variable region subgroup. These subgroups of the heavy chain are apparently shared by the γ and μ classes, whereas light chain subgroups are peculiar to the class of light chain.

The Gm groups f^+ and z^+ are probably related to an arginine-lysine replacement in the constant region of the Fd-fragment [4,7], and are not correlated with the subgroups based on variable region sequences, since although Cor and Daw are Gm(z^+) and Ste and Eu are Gm(f^+) the μ chain (Ou) does not express Gm determinants, but can be grouped with Daw and Cor from its variable region sequence.

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