PARTIAL AMINO ACID SEQUENCE OF THE VARIABLE REGION OF A MOUSE γG2a IMMUNOGLOBULIN HEAVY CHAIN. EVIDENCE FOR THE EXISTENCE OF A THIRD SUB-GROUP OF VARIABILITY FOR THE HEAVY CHAIN POOL

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1. Introduction

Subgroups of variability have been described for both the light [1–3] and the heavy chains [4–8] of human immunoglobulins. In a previous publication [9], we have reported the isolation and characterization of CNBr fragments of murine plasmocytoma protein MOPC 173. Ten fragments, H1 to H10 were assigned to the heavy chains, and provisionally ordered. Fragments H1, H2, and H3 contained 34, 48 and 21 amino acid residues, respectively, and therefore accounted for the major part of the variable region of the heavy chains [4, 6, 8].

In the present communication, we report the sequence of fragments H1 and H3, linked together through a disulfide bond, and representing a total of 55 residues, thus covering approximately half of the Hv region.

2. Materials and methods

Preparation of MOPC 173 plasmocytoma protein and that of its CNBr derived fragments has been previously described [9]. Tryptic and chymotryptic peptides were separated by ion-exchange chromatography on Dowex 50 × 2 and further purified by high voltage paper electrophoresis, whenever necessary. Amino acid sequence was determined by the Edman-dansylation method [10, 11].

Sequences were compared as suggested by Fitch [12] by calculating the minimum number of mutations required for every pair of amino acids in the homologous stretches.

3. Results

The NH_2-terminus of the heavy chains was identified as glutamic acid; so was that of the H1 fragment which was thus assigned to the NH_2-terminal portion of the heavy chain. Four tryptic peptides were isolated from fragment H1, in agreement with its lysine and arginine content. The amino acid sequence directly determined on residues 1 to 8 of fragment H1 imposed the order H_1T_1–H_1T_2. Peptide H_1T_4 contained HSR *, therefore the overall order was H_1T_1–H_1T_2–H_1T_3–H_1T_4 as indicated on fig. 1. Analysis of the chymotryptic peptides provided additional evidence for the correctness of this conclusion.

Amino acid sequence of residues 1 to 10 of fragment H_3 was directly determined and imposed the order of the tryptic peptides H_3T_1–H_3T_2–H_3T_3a. As H_3T_5 contained an HSR residue, the overall

* *Abbreviation:* HSR = homoserine.

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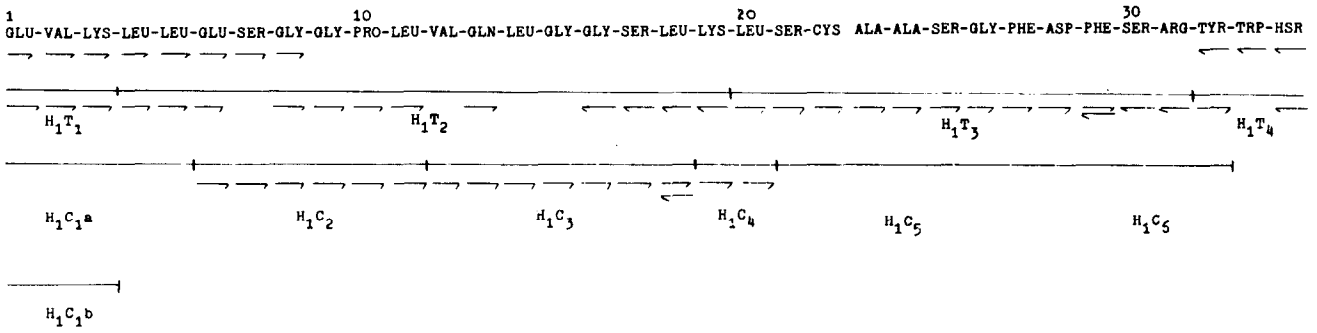

Fig. 1. Amino acid sequence of fragment H_1, as determined by the Edman-dansylation technique (——→) and by carboxypeptidase (A + B) digestion (←——). H_1T_1 to H_1T_4: tryptic peptides and H_1C_1a to H_1C_6: chymotryptic peptides.

order must be that presented on fig. 2. Free tyrosine eluted with chymotryptic peptide H_3C_1 upon ion-exchange chromatography on Dowex 50 X 2 and further separation was obtained by high voltage paper electrophoresis.

4. Discussion

Most heavy chains analyzed so far had blocked NH_2-termini. MOPC 173 heavy chain and a human γG4 myeloma protein (Vin) were found to possess glutamic acid as NH_2-terminal residue [13]. When the sequence of MOPC 173 H_1 fragment was compared with the corresponding sequence of human myeloma protein Vin (fig. 3), a strong homology was observed, since 67% of the positions (23 out of 34) were identical. Two subgroups of variability have already been reported for the human heavy chains [4–7]. Within the subgroup containing proteins Daw, He and Ou (fig. 3) 75% of the positions were identical when the homologous portion of 34 residues was considered. Homology was maximized, however, when residues adjacent to position 30 were not taken into consideration, perhaps because of the possible proximity of this area (close to the first intrachain disulfide bond) to the antibody active site. This situation would thus seem to parallel that described for the light chains [2]. Residues 1 to 27 (or 1 to 24 for the subgroup containing Ste, were tested for homology by expressing the minimum number of mutations required per codon [12]. It may be observed

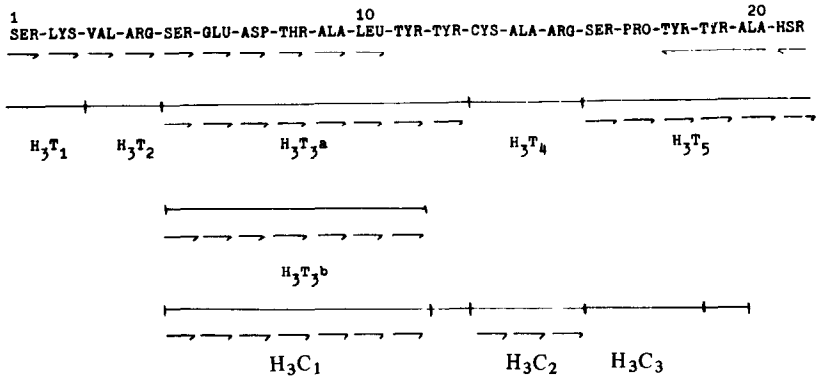

Fig. 2. Amino acid sequence of fragment H_3, as determined by the Edman-dansylation technique (——→) and by carboxypeptidase (A + B) digestion (←——). H_3T_1 to H_3T_5: tryptic peptides and H_3C_1 to H_3C_3: chymotryptic peptides.

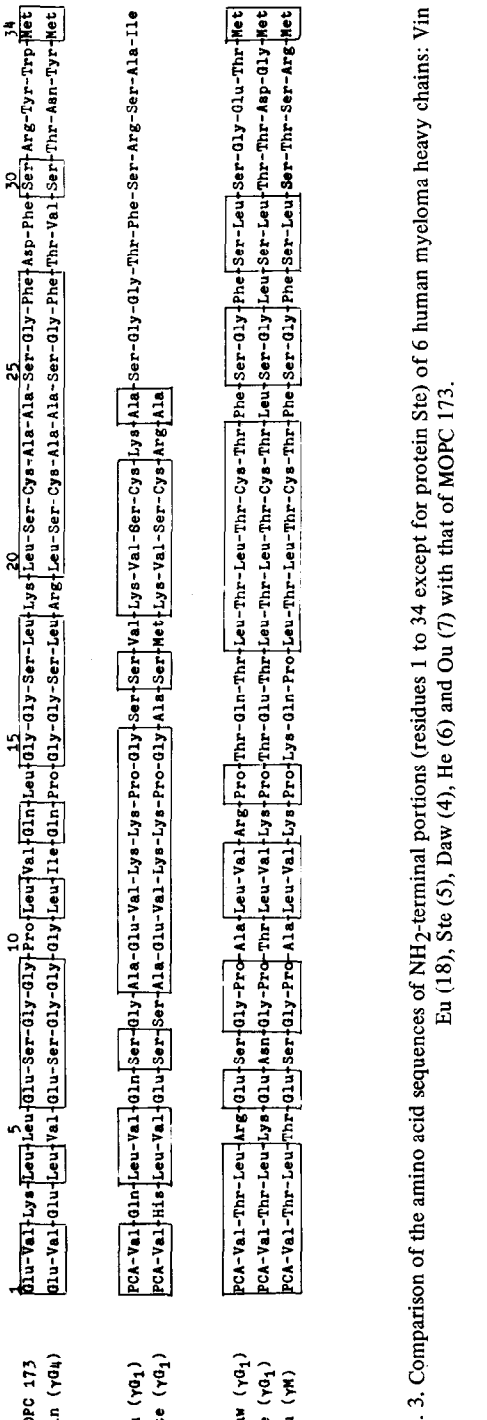

Fig. 3. Comparison of the amino acid sequences of NH_2-terminal portions (residues 1 to 34 except for protein Ste) of 6 human myeloma heavy chains: Vin (12) Eu (18), Ste (5), Daw (4), He (6) and Ou (7) with that of MOPC 173.

Table 1

Comparison of residues 1 to 27 of one murine plasmocytoma heavy chain with the corresponding sequences of 6 human myeloma proteins.

	MOPC	Vin	Eu	Ste	He	Daw	Ou
MOPC	0	0.26	0.70	0.75	0.74	0.67	0.70
Vin		0	0.63	0.67	0.81	0.74	0.74
Eu			0	0.25	0.85	0.89	0.85
Ste				0	0.96	0.96	0.92
He					0	0.26	0.30
Daw						0	0.15
Ou							0

Figures represent the minimum number of mutations required per codon, as suggested by Fitch [11].

(table 1) that the calculated figures were below 0.30 when human heavy chains pertaining to the same subgroup were compared (i.e. Eu and Ste or Daw, He and Ou) whereas values over 0.63 were consistently observed whenever heavy chains from different subgroups were compared. From the calculated value of 0.26 resulting from comparison between the human heavy chain Vin and the homologous sequence of the murine MOPC 173 protein, it seems reasonable to propose the following:

(a) Proteins Vin (human γG4) and MOPC 173 (murine γG2a) allows us to define a third subgroup of variability for the heavy chains.

(b) at least for this subgroup, and for the considered stretch of sequence, a strong selection pressure does exist which imposes the conservation of large segments of the variable region of the heavy chains. This observation supports the idea that selection of antibody variability occurred prior to mouse-human divergence in the course of evolution [14, 15].

(c) In agreement with the translocation hypothesis [6, 7, 16, 17] our results favor the existence of a common pool of variability for the heavy chains, as opposed to the situation described for the light chain κ and λ systems. Care should be taken however in making such a generalization, in view of the limited number of sequences reported so far.

A different situation was observed when homology was investigated with fragment H_3. Since H_3 was

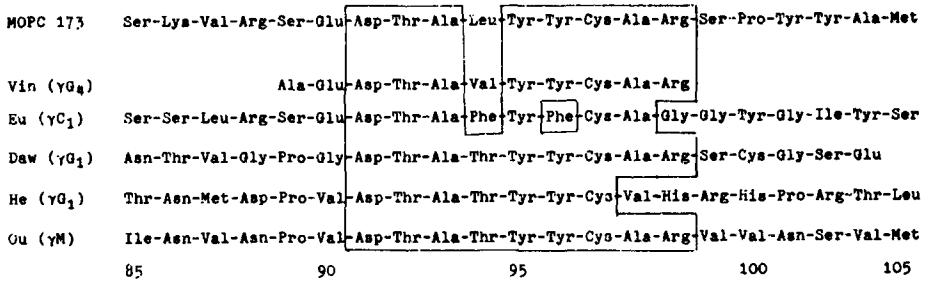

Fig. 4. Comparison of the amino acid sequences between residues 85 and 105 (on numbering) of five human myeloma and MOPC 173 heavy chains. Origins of the data concerning the human chains are mentioned in the legend of fig. 3.

linked to H_1 by a disulfide bond, we proposed [9] by analogy with other immunoglobulins, that this fragment covered region 83–103 of the MOPC 173 heavy chain. When a limited number of deletions were taken into account in order to maximize homology, this position of H_3 appears to be valid (fig. 4).

The homology pattern that characterizes the various sequences presented on fig. 4 is strikingly different from that discussed for the terminal portions of the heavy chains. It may be observed, as already pointed out by Press and Mogg [4] that classification of the different heavy chains into subgroups becomes hardly justified in this region, since residues 91–99 (Ou numbering) remain practically constant throughout the series, whereas adjacent residues exhibit extensive variability. The presence of quasi-invariant tyrosyl residues in this region, already apparent from affinity labeling experiments [18] taken together with the extreme variability observed in the vicinity makes it consistent that this portion of the heavy chains might contribute to the antibody active site.

Acknowledgments

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References

[1] H.D.Niall and P.Edman, Nature 216 (1967) 262.
[2] C.Milstein, Nature 216 (1967) 330.
[3] V.B.Langer, M.S.Kayne and N.Hilschmann, Z. Physiol. Chem. 349 (1968) 945.
[4] E.M.Press and N.M.Hogg, Nature 223 (1969) 807.
[5] C.E.Fisher, W.H.Palm and E.M.Press, FEBS Letters 5 (1969) 20.
[6] B.A.Cunningham, M.N.Pflumm, U.Rutishauser and G.M.Edelman, Proc. Natl. Acad. Sci. U.S. 64 (1969) 997.
[7] M.Wilker, H.Kohler, T.Shinoda and F.W.Putnam, Science 163 (1969) 75.
[8] G.M.Edelman, B.A.Cunningham, W.E.Gall, P.D.Gottlieb, U.Rutishauser and M.J.Waxdal, Proc. Natl. Acad. Sci. U.S. 63 (1969) 78.
[9] A.Bourgois and M.Fougereau, European J. Biochem. 12 (1970) 558.
[10] W.R.Gray, Methods Enzymol. 11 (1967) 469.
[11] R.R.Woods and K.T.Wang, Biochim. Biophys. Acta 133 (1967) 369.
[12] W.M.Fitch, J. Mol. Biol. 16 (1966) 9.
[13] J.R.L.Pink and C.Milstein, FEBS Symposium 15 (1969) 177.
[14] G.M.Edelman and W.E.Gall, Ann. Rev. Biochem. 38 (1969) 415.
[15] C.Milstein and J.R.L.Pink, Prog. Biophys. Mol. Biol. (in press).
[16] W.J.Dreyer and J.C.Bennett, Proc. Natl. Acad. Sci. U.S. 54 (1965) 864.
[17] E.S.Lennox and M.Cohn, Ann. Rev. Biochem. 36 (1967) 365.
[18] S.J.Singer and N.O.Thorpe, Proc. Natl. Acad. Sci. U.S. 60 (1968) 1371.