CROSSING OVER AND ANTIBODY DIVERSITY: THE SEQUENCE OF A NEW HUMAN κI LIGHT CHAIN

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Received 5 December 1972

1. Introduction

Immunoglobulin light chains isolated from monoclonal myeloma proteins from man are made up of two sections of approximately equal length. Unlike the constant, C-terminal half of κ light chains, the N-terminal half is different in each individual. In spite of this variability a high degree of similarity among some of the light chains has been found and this has led to the classification of the human κ chains into 3 families or subgroups κI, κII and κIII [1]. Chains belonging to the same subgroup have generally the same residue at certain defined positions along the chain, thereby defining one basic sequence for each of these subgroups [1]. Basic sequences represent the commonest residues found at given positions in most, if not all, the members of the subgroup. There are also 'hypervariable' positions [1] and, in addition, some positions are occupied by either one of two residues occurring with about equal frequency. These latter positions when tabulated [2], appeared to be linked. These were positions 24, 50, 56, 73, 83, 92 and 100, their nature is shown in table 1. In at least one case (position 24, where either glutamine or arginine is found in proteins of the κI type) both forms were found in the sera of all normal individuals. This as well as the rest of the above mentioned linked substitutions define two distinct populations of molecules which appear to be coded by non-allelic genes [3]. It was proposed that the two above mentioned populations are two subgroups of κI, κIa and κIb [2].

Myeloma protein Car was selected for further sequence determinations in order to help define the two populations and this paper presents the sequence of this protein (fig. 2). A full report will appear elsewhere.

2. Results and discussion

The myeloma protein Car was carboxymethylated [4] and fractionated on a Sephadex G-100 column in 5% v/v formic acid. The separated light chains were fully reduced and carboxymethylated with iodo [^{14}C]acetate and hydrolysed with trypsin or pepsin [3]. The sequence shows that Car belongs to the κIb subgroup. It is the only protein of the κI subgroup which presents a substitution at position 20. This substitution corresponds to a single base mutation. Another "low frequency variant" [5] found in this protein is at position 51. It also presents substitutions at positions 10, 28, 34, 46, 81, 94 and 105 when compared with κI basic sequence. All these substitutions could be the result of one base mutation from the basic sequence except for position 34 which requires a two base substitution.

Table 1 compares the subgroups of κI basic sequences brought up to date. At position 73 the κIb protein Ou [13] contains a phenylalanine which is one of the residues of the basic sequence κIa at that position. On the other hand, proteins Bel [7] and Scw [10] of the κIa subgroup have position 73 substituted by leucine, which is the amino acid residue of the basic sequence κIb. A similar situation applies to protein Bel at position 83. An extreme situation is shown by protein Bi [14] since from the nature of

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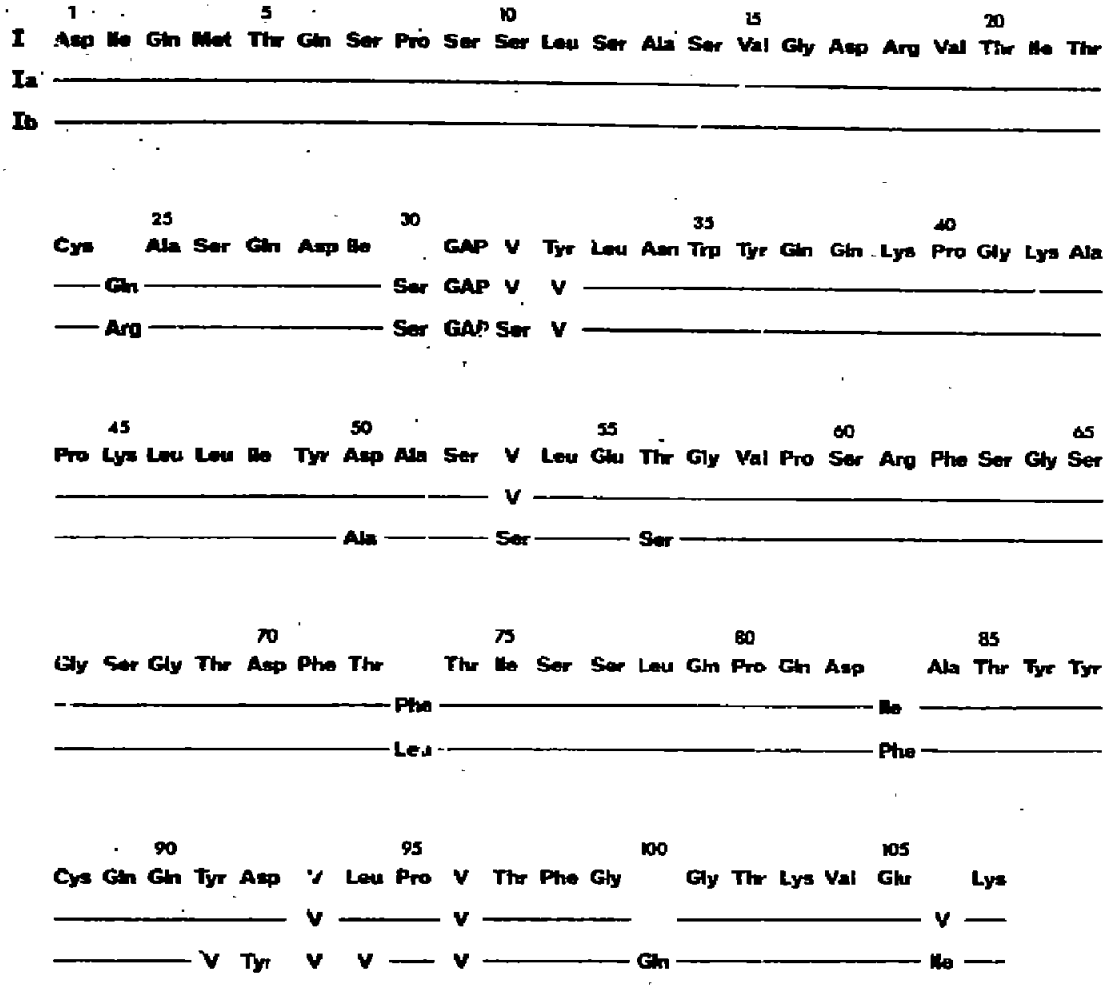

Fig. 1. Sequences of κ-chain subgroups. κI basic sequence was taken from Milstein and Munro [4]. κIa and κIb basic sequences are defined to accommodate recent data. A line means that the residue is the same as that of κI. When none of the several variants occur with a frequency of at least 2:1 over any of the others, this is shown with a V. When two of several variants occur with approximately the same frequency the position is left blank.

residues 24 and 50 it should be placed in the κIa subgroup; it was included in the κIb on the basis of the minimal number of required substitutions. The presence of group-specific residues in proteins of the other subgroup suggested the possibility of crossing over between genes of different subgroups.

It is generally accepted that there is no detectable cross over between κI and κIII [2]. It is possible to calculate whether the frequency of "coincident residues" is greater in the case of the closely related κIa and κIb than between κI and κIII subgroups. Using the data shown in tables 2 and 3 it is possible to calculate:

a) the frequency of variants of the κI basic sequence which coincide with κII or κIII basic sequences. The frequency is 3/10 (0.300) in each case.

b) the frequency of variants of κIII coinciding with κI or κII, which are 3/12 (0.250) and 2/8 (0.250), respectively. No table containing the group specific variants of κII has been made since there are insufficient sequence data in this subgroup.

The values mentioned above are not significantly different from the value found for the frequency of variant residues occurring in κIa and κIb which correspond with those in κIb and κIa, respectively, which is 7/33 (0.212). It has been suggested that the fre-

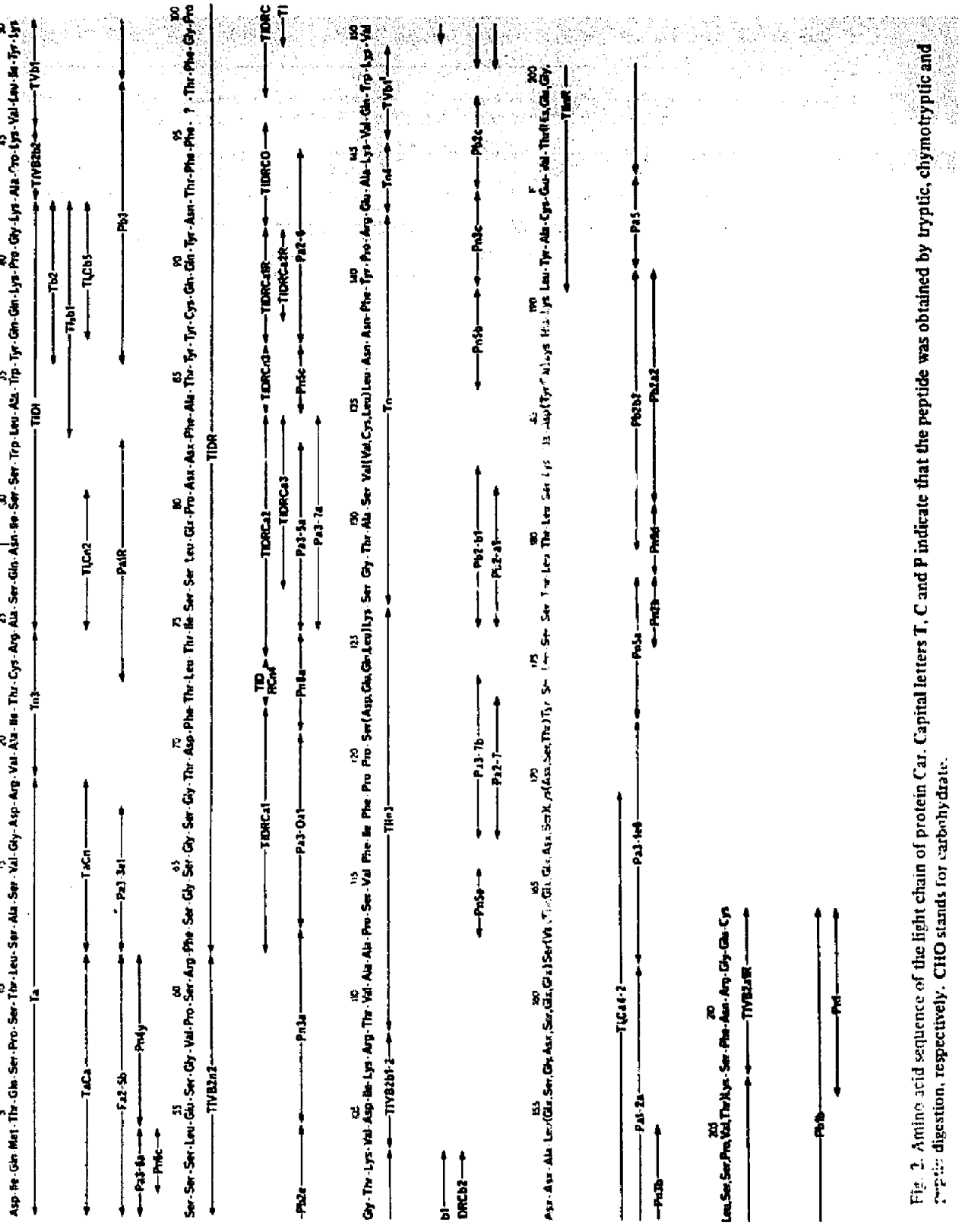

Fig. 2. Amino acid sequence of the light chain of protein Car. Capital letters T, C and P indicate that the peptide was obtained by tryptic, chymotryptic and peptic digestion, respectively. CHO stands for carbohydrate.

Table 1
Subgroups of κI basic sequence.

	Protein	24	50	56	73	83	92	100	References
κIa	(1)	Gln	Asp	Thr	Phe	Ile	Asp		
	Ag	—	—	—	—	—	—	Gln	[6]
	Bel	—	—	—	Leu	Phe	—	Gly	[7]
	Roy	—	—	Ala	—	—	—	Gly	[8]
	Au	—	—	Ser	—	—	—	Gln	[9]
	Scw	—	Gly	—	Leu	—	—	Gln	[10]
κIb	(1)	Arg	Ala	Ser	Leu	Phe	Tyr	Gln	
	Dee	—	—	—	—	—	—	Pro	[3]
	Eu	—	Lys	—	—	—	Asx	Glx	[11]
	Hau	—	—	—	—	—	—	—	[12]
	Ou	—	—	—	Phe	—	—	Glx	[13]
	Car	—	Lys	—	—	—	Asn	Pro	This paper
	Bi	Gln	Asp	Ile	—	—	—	—	[14]

(1) Residues of basic sequence.
A line means that the numbered residue of the protein and the basic sequence are the same. This line is interrupted when a difference occurs and the variant residue is included.

Table 2
Group specific residues of κIa and κIb.

Positions		Frequency of variants				
		Coinciding with basic sequence				Not coinciding
	κIa / κIb	κIa	κIb	κII	κIII	
24	Gln	–	–	–	–	–
	a)b) Arg	1/6	–	–	–	–
50	Asp	–	–	c	c	Gly 1/5
	Ala	1/6	–	c	c	Lys 2/6
56	b) Thr	–	1/5	1/5	–	Ala 1/5
	a) Ser	–	–	–	–	Ile 1/6
73	Phe	–	2/5	2/5	2/5	–
	a)b) Leu	1/6	–	–	–	–
83	Ile	–	1/5	–	1/5	
	b) Phe	–	–	–	–	–
92	Asp	–	–	–	–	–
	Tyr	–	–	–	–	Asn 1/6

a) Same residue as the one of κII basic sequence.
b) Same residue as the one of κIII basic sequence.
c) No assigned residue in basic sequence.

Table 3
Group specific residues of κIII.

Positions	Frequency of variants		
	Coinciding with		Coinciding with neither
	κI	κII	
17. b) Glu	1/4	–	–
20. a) Thr	–	–	1/4
28. Ser	–	–	1/4
29. Val	–	1/4	1/4
30. Ser	–	–	1/4
45. Arg	1/4	–	–
58. Ile	–	–	1/4
60. Asp	–	–	1/4
79. Gln	–	1/4	–
92. Gly	–	–	1/4
104. b) Leu	1/4	–	–

a) Same residue as the one of κI basic sequence.
b) Same residue as the one of κII basic sequence.

quency of crossing over will be higher between closely similar genes than between less similar ones. There are 6 amino acid differences between the basic sequences of κIa and κIb, 29 between κI and κII and 24 between κI and κIII. In spite of this, there is no detectable increase in frequency of apparent crossing over as measured by the occurrence of group specific residues characteristic of another subgroup. However the frequency of such apparent cross overs is higher than would be expected from random single-point mutation, which is of the order of 1/6 (0.167). It is possible, as suggested by others [15], that this departure from randomness is mainly due to selective pressures for the maintenance of a correct tertiary structure.

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