HOMOLOGIES IN THE POSITION OF CYSTEINE RESIDUES OF K AND L TYPE CHAINS OF HUMAN IMMUNOGLOBINS

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Two antigenic types of light chains of human immunoglobulins have been recognized : K and L. These peptide chains, called κ and λ respectively, show some similarity in their structure : the COOH-terminal half is identical in all the chains of the same antigenic type, whereas the NH_2-terminal half is variable (Hilschmann and Craig, 1965; Cioli and Baglioni, 1966). Peptides obtained from the COOH-terminal half are thus called common peptides, whereas peptides from the NH_2-terminal half are called distinctive peptides (Baglioni and Cioli, 1966).

The amino acid sequence of some κ light chains is known (Titani et al., 1965), whereas partial amino acid sequences have been reported for some λ chains (Hood et al., 1966; Milstein, 1966). These sequences have shown a surprising homology between the two types of light chains, both in the variable and in the common region. The present communication describes some sequence studies on tryptic peptides of a λ chain. These studies have made it possible to localize two of the cysteine residues of the λ chain, besides the two that have been previously localized by Milstein (1966). It appears

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that the cysteines which form intrachain -S-S- bonds are localized in the same positions in κ and λ chain. This has suggested that the light chains of immunoglobulins have a very similar tertiary structure.

MATERIALS AND METHODS

Bence Jones proteins were obtained from the sources previously outlined (Baglioni and Cioli, 1966) and were purified accordingly. The proteins were aminoethylated and digested with trypsin, as previously described (Baglioni et al., 1965). The tryptic digests were fractionated by gel filtration on Sephadex G-25, using 1% formic acid as eluant (Baglioni C. and Alescio-Zonta L., in preparation). The fractions eluted from Sephadex were then separated by chromatography on Dowex 50 according to Jones (1964). Peptides which were not found completely pure were further isolated by high voltage ionophoresis or chromatography.

Amino acid analyses were performed on a Beckman 120 C Analyzer. Digestion with proteolytic enzymes was carried out as previously described (Baglioni, 1962). The products of hydrolysis were separated by high voltage ionophoresis at pH 4.7, as described by Naughton and Hagopian (1962). The NH_2-terminal amino acids were determined by the method of Sanger, as described by Frankel-Conrat et al. (1955). Peptides were detected by ninhydrin, by chlorination and by specific staining reactions (Baglioni et al., 1965).

RESULTS AND DISCUSSION

All the tryptic peptides have been isolated from a Bence Jones protein (BJ 98) of type L. The peptides have been analyzed for the

amino acid composition and striking homologies were noted with known peptides of the κ chain. Peptide S1D1, which was only detected by chlorination or by its reaction with the platinic iodide reagent for reduced sulfur, had a composition similar to that of the NH_2-terminal peptide of K type proteins (Titani et al., 1965). Peptide S1D1 was tentatively identified with the NH_2-terminal peptide of protein BJ 98 because of the presence of a blocked α-amino group. Protein BJ 98 showed a blocked α-amino group, like the majority of L type proteins that have a blocked glutamic acid residue - presumably pyrrolidone carboxylic acid - at the N-terminus (Hood et al., 1966).

A striking homology of peptide S1D1 with peptides, reported by Hood et al. (1966) to be at the N-terminus of λ chains, was observed. A partial sequence of peptide S1D1 was thus determined by means of partial hydrolysis with proteolytic enzymes (Table I). The partial sequence established confirmed the homology noticed by Hood et al. (1966) between the N-terminus of the κ chain and that of the λ chain, and extended the sequence to the first cysteine residue of the λ chain. As reported by Hood et al. (1966), the NH_2-terminal sequence of the λ chain is shorter by one amino acid than that of the κ chain. The cysteine residue that occupies position 23 in the κ chain is found in position 22 in the λ chain. The homologies between these two peptide chains thus appear considerable also in the variable region of the chain. This may indicate that not all the residues of the variable region are subject to variation; some of the amino acid residues which are homologous in the two chains show little or no variability. Whether the presence of some crucial residues is a structural requirement of

TABLE I

Amino acid sequence of the NH_2-terminal tryptic peptide of the chain.

Peptide																				
BJ 98-T 1	*Glp	(Ser	Val	Leu	Thr	Glx	Pro	Pro	Ser	Val	Ser	Ala	Ala	Asx	Gly	Glx	Ala	Val	Thr	Ser	Ilu)Cys
T 1-X 1					*Thr	(Glx	Pro	Pro	Ser	Val	Ser	Ala	Ala	Asx	Gly	Glx	Ala	Val	Thr	Ser	Ilu)Cys
T 1-Pe 1					Thr	(Glx	Pro	Pro	Ser	Val	Ser	Ala	Ala	Asx	Gly	Glx	Ala)				
T 1-Pe 5					Thr	(Glx	Pro	Pro	Ser	Val	Ser	Ala)									
T 1-Pa 4					Thr	(Glx	Pro	Pro	Ser	Val	Ser)										
T 1-Pa 5											Ser	(Ala	Ala	Asx	Gly	Glx	Ala	Val	Thr	Ser	Ilu)Cys
T 1-Pe 1												Ala	(Asx	Gly	Glx	Ala)					
T 1-X 3																(Glx	Ala	Val)			
T 1-Pe 7																		Val	(Thr	Ser	Ilu)Cys
T 1-Pe 6																				Ser	.Ilu .Cys

BJ 98-T 1 (partial sequence)									10										20		
	Glp	(Ser	Val	Leu)	Thr	(Glx	Pro	Pro	Ser	Val	)Ser	.Ala	.Ala	(Asx	Gly)(Glx	Ala)Val	.Thr	.Ser	.Ilu	.Cys
/HBJ 2 (λ)	Glp.	Ser.	Ala.	Leu.	Thr.	Gln.	Pro.	Pro.	Ser	Ala.	Ser.	Gly.	Ser.	Pro.	Gly.	Gln.	Ser.	Val.	Thr.		
/AG (K)	Asp.	Ilu.	Gln.	Met.	Thr.	Gln.	Pro.	Ser.	Ser.	Ser.	Leu.	Ser.	Ala.	Ser.	Val.	Gly.	Asp.	Arg.	Val.	Thr.	Ilu.	Thr.	Cys
										10									20		

* Glp indicates a glutamic acid residue with a blocked α-amino group, probably pyrrolidone carboxylic acid. Glx and Asx indicate glutamic acid or glutamine and aspartic acid or asparagine respectively. Amino acids which have been determined to be at the NH_2-terminal position of a peptide are underlined.

/The sequence of a λ chain (Hood et al., 1966) and of a K chain (Titani et al., 1965) are reported for comparison.

the light chains of immunoglobulins, or whether some residues are less subject to variation, is not known. The mechanism which produces variability of the NH_2-terminal half of light chains being still obscure, one does not know whether chains which change in one of the structurally important amino acid residues are not synthesized. Alternatively, the mechanism which produces variability may change only some amino acid residues in a specific way.

Two more peptides which have been obtained from protein BJ 98 have been located by homology with the amino acid sequence of the κ chain. The complete amino acid sequence of peptide S5D3, which contains a cysteine, has been determined (Table II).

TABLE II
Amino acid sequence of peptide BJ98S5D3

BJ98S5D3	*Ala (Thr Leu Val)Cys
Peptides obtained by chymotryptic digestion:	
X-1	Val.Cys
X-2	Ala. Thr
X-3	(Leu Val Cys)
Amino acid sequence:	Ala. Thr. Leu.Val. Cys

*Amino acid residues which have been determined to be at the NH_2-terminal position of a peptide are underlined.

When the amino acid sequence reported by Milstein (1966) for a chain is considered, one sees that peptide S5D3 is the only peptide isolated from BJ 98 that can presumably follow in the sequence, since it shows three identical amino acids out of five with the homologous sequence of the κ chain. This peptide has been observed in all the peptide maps of Bence Jones proteins of type L so far examined (peptide λ 9;

TABLE III

Comparison of the amino acid sequence of the κ and λ chain around the "common" cysteine residue 134.

		110										120								
κ*	Thr.	Val.	Ala.	Ala.	Pro.	Ser.	Val.	Phe.	Ile.	Phe.	Pro.	Pro.	Ser.	Asn.	Glu.	Gln.	Leu.	Lys.		
λ			Ala.	Ala.	Pro.	Ser.	Val.	Thr.	Leu.	Phe.	Pro.	Pro.	Ser.	Ser.	Glu.	Glu.	Leu.	Gln.		
tryptic peptide								S2D1•												
			130													140				
	Ser.	Gly.	Thr.	Ala.	Ser.	Val.	Val.	Cys.	Leu.	Leu.	Asn.	Asn.	Phe.	Pro.	Tyr.	Arg				
	Ala.	Asn.	Lys.	Ala.	Thr.	Leu.	Val.	Cys.	Leu	(Leu	Gln	Gly	Thr	Pro	Val)	Lys				
				S5D3						S4D2E3										

*The amino acid sequence of protein Ag reported by Titani et al. (1965) is reproduced.

• Peptide S2D1 has been obtained from protein BJ 98; the amino acid composition of this peptide coincides with that of a partial sequence of a λ chain, which is reproduced from Milstein (1966).

Baglioni and Cioli, 1966) and it is thus a "common" peptide. Another peptide, S4D2E3, has been found which is homologous to a peptide of the K chain and presumably follows peptide S5D3 in the sequence (Table III).

Positioning of peptide S5D3 makes it possible to establish that all the cysteines of the common region of the λ chain occupy homologous positions with the cysteines of the K chain. This indicates that these chains have a common tertiary configuration, possibly characterized by two equally-spaced intrachain disulphide bridges, one in the variable and one in the common half of the molecule, and one interchain disulphide bridge that links the light to the heavy chain (Milstein, 1964). The homologies between the two types of light chains, as well as the homologies between the light and the heavy chains (Hill et al., 1966) indicate that the corresponding genes have evolved from a common ancestor gene through a process of gene duplication and independent evolution, similar to that suggested by Ingram (1961) to account for the homologies in the amino acid sequence of human hemoglobin chains.

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