Amino Acid Sequence of the Variable Region of the Light (λ) Chain from Human Myeloma Cryoimmunoglobulin IgG Hil†

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ABSTRACT: We have determined the complete amino acid sequence of the variable region of the light (λ) chain from a human myeloma cryoimmunoglobulin (IgG Hil), the Fab fragment from which has been previously crystallized. The presence of unblocked α-amino terminal residue and the isolation of a CNBr fragment starting at position 46 and of a maleylated tryptic fragment spanning residues 61 to 189 provided three suitable starting points for automatic Edman degradation. In addition, tryptic peptides and chymotryptic subpeptides covering the whole extension of the light chain were obtained and characterized to further verify the sequence of the variable region and the established sequence of the constant region. The proposed sequence of the variable region indicates that it may be assigned to subgroup III. Positions 152 (serine) and 189 (arginine) correspond to the isotypic markers $Kern^-$ and Oz^-, respectively. In addition, a novel substitution has been detected in the constant region where at position 155 isoleucine replaces the usually occurring valine.

Studies on the amino acid sequence and the three-dimensional structure of the Fab[1] fragment from a human immunoglobulin, IgG New, have been reported (Poljak et al., 1972, 1973, 1974; Chen & Poljak, 1974). A second crystalline Fab fragment, obtained from human myeloma cryoimmunoglobulin IgG Hil, is under study in this laboratory (Humphrey et al., 1969). A specific aim of this study is to correlate the amino acid sequence at the hypervariable regions of IgG Hil with the conformation of its combining site. Comparison of this site with that of IgG New will contribute to our understanding of the relation between primary and tertiary structure and, in particular, to that between amino acid sequence and antibody specificity. A necessary step in these studies is the determination of the amino acid sequences of the heavy (H, γ) and light (L, λ) polypeptide chains of IgG Hil. In this paper we report on the amino acid sequence of the variable amino terminal region of the L (λ) chain of IgG Hil (V_L 107 amino acid residues long) (Figure 1). In addition, the sequence of the constant, C-terminal region (C_L, 105 residues) has been verified by compositional analysis of the corresponding tryptic peptides.

Materials and Methods

Preparation of L Chain Hil. Human myeloma immunoglobulin Hil (IgG1, a cryoglobulin, Gm (1+, 3−, 4−, 5−), λ L chain) was purified from serum as previously described (Rossi & Nisonoff, 1968). Preparative separation of H and L chains was carried out after mild reduction and carboxymethylation of purified IgG as described before for IgG New (Chen & Poljak, 1974). Totally reduced and aminoethylated L chain was prepared by reduction in 8 M guanidinium hydrochloride, pH 8.5, 0.005 M EDTA, 0.1 M dithiothreitol for 4 h at 40 °C. Aminoethylation was carried out as described (Chen & Poljak, 1974). Alternatively, carboxymethylation with iodoacetic acid was performed after complete reduction. Analytical isoelectric focusing of mildly reduced and alkylated L chain was performed on acrylamide gel slabs (Awdeh et al., 1968) in 6 M urea, with an ampholine gradient of pH 3.5–10.0. It revealed three major, regularly spaced bands focusing in the region of pH 5.3–5.6. Human and murine monoclonal immunoglobulins and their polypeptide chains are known to give such patterns (Awdeh et al., 1968). The L chain material appeared free from H chains when analyzed by this technique.

Sequence Determination. Automated Edman degradations (Edman & Begg, 1967) were carried out using a 890C Beckman sequencer and the Beckman protein quadrol program no. 122974.

Identification of Pth-Amino Acids. Three methods were regularly used to identify the products of sequential Edman degradations: thin-layer chromatography (Summers et al., 1973), high-pressure liquid chromatography (HPLC, Zimmerman et al., 1977), and HI hydrolysis followed by amino acid analysis (Smithies et al., 1971). Due to its reproducibility, high sensitivity, speed and ease of operation, HPLC was the primary procedure used for identification and quantitation of Pth-amino acids. A Du Pont Model 830 high performance liquid chromatograph, fitted with a 0.46 × 25 cm Zorbax ODS column with 14 000 theoretical plates, was operated at 62 °C under a N_2 pressure of 1000 psi and a flow rate of 1.6 mL/min. Pth-amino acid standards (Pierce Chemical Co.) were dissolved in methanol (4 nmol/μL) and a sample containing 1 nmol of each Pth standard was loaded onto the column. The isocratic procedure of Zimmerman et al. (1977) was used, except that the solvent composition was 0.1 N sodium acetate (pH 4.5)–acetonitrile (58:42, v/v). The increased concentration of the acetate buffer over that proposed before (0.01 N, Zimmerman et al., 1977) gave improved separations under our experimental conditions (Figure 2). Other concentrations did not work as well. All Pth-amino acids were eluted and separated within 9 min with good resolution (Figure 2), except that Pth-Ser and Pth-Gln overlapped. Pth-Asp moved with the solvent front (methanol). These ambiguities were resolved by use of the thin-layer chromatography and amino acid analysis after HI hydrolysis. The elution times of some of the peaks

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[1] Abbreviations used: those for immunoglobulins, their polypeptide chains and fragments are as recommended (1964), *Bull. W.H.O. 30,* 447; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; HPLC, high performance liquid chromatography; Pth, phenylthiohydantoin.

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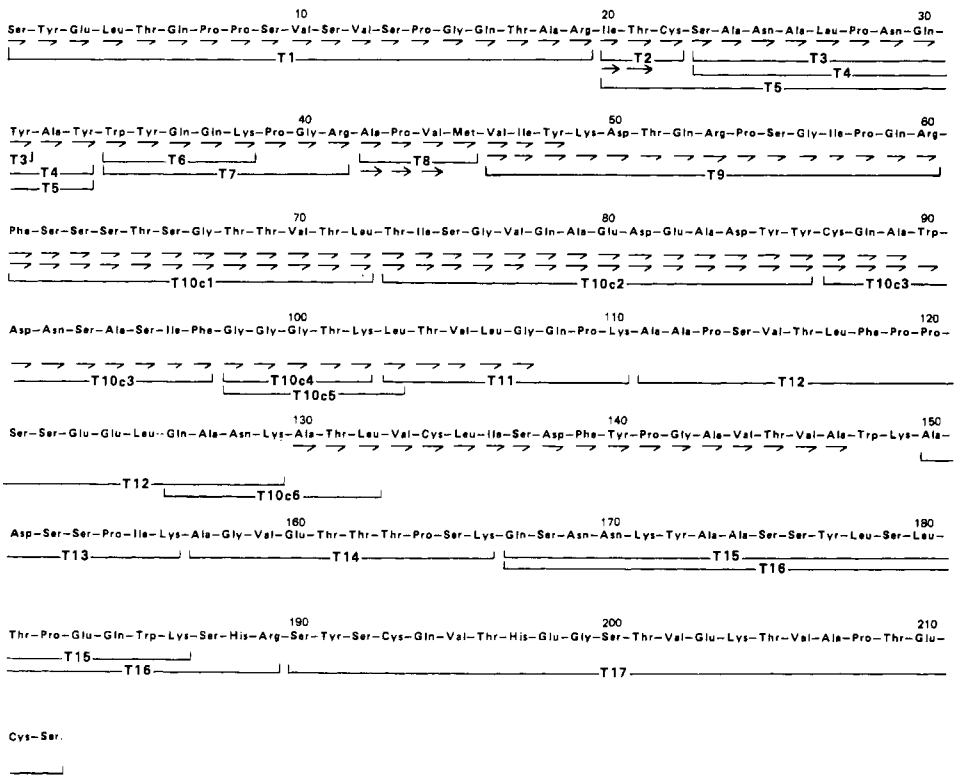

FIGURE 1: Amino acid sequence of the L(λ) chain Hil. Half arrows indicate residues that were determined by automatic Edman degradation of (a) the whole L chain (positions 1-48), (b) the CNBr fragment LI (positions 46-89), (c) a tryptic peptide of the maleylated LI (positions 61-107), and (d) another tryptic peptide of the maleylated LI (positions 130-147). Tryptic peptides (T) and their chymotryptic subpeptides (Tc) that were characterized are indicated. Full arrows represent amino acids of tryptic peptides T2 and T8 that were identified by dansyl-Edman techniques. The sequence of the tryptic peptides of the C region (residues 108-212) is assumed to be that established for other human λ chains.

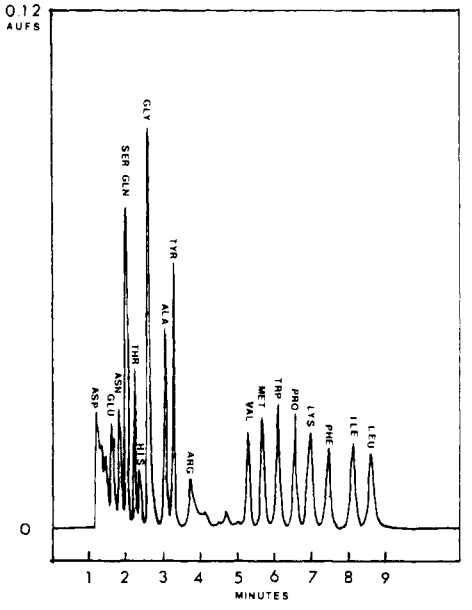

FIGURE 2: Pth-amino acid separation by HPLC. Twenty microliters of a mixture containing 1 nmol of each of 19 Pth-amino acids was loaded at zero time on a 0.46 × 25 cm Zorbax ODS column. They were eluted with a mobile phase consisting of 0.1 M sodium acetate (pH 4.5)-acetonitrile (58:42, v/v).

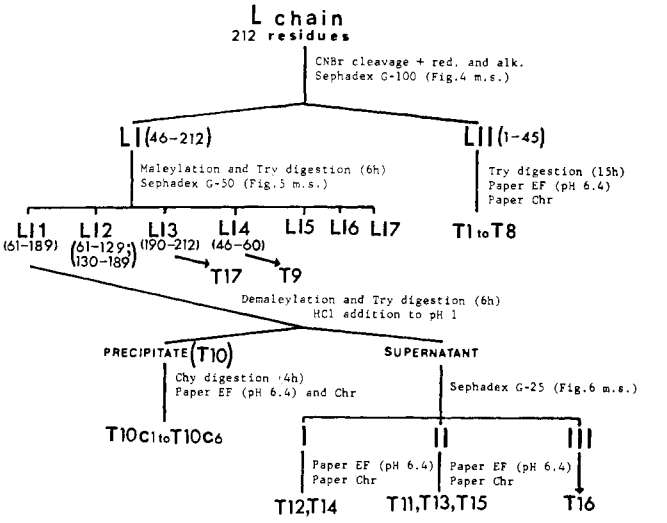

FIGURE 3: Schematic representation of the isolation and purification of tryptic peptides and chymotryptic subpeptides of L-chain Hil. Abbreviations used in this figure are: alk., alkylation; Chr, chromatography; Chy, chymotrypsin; EF, electrophoresis; red., reduction; Try, trypsin. Numbers in parentheses refer to the amino acid residue number in the final sequence (see Figure 1) T1 to T17 are designations for tryptic peptides as shown in Figure 1.

illustrated in Figure 2 shifted systematically after prolonged use of the column. Pth-amino acid standards were run at frequent intervals (between runs of unknown samples) to verify elution times and unambiguously identify unknown Pth-amino acids. Due to the higher salt concentration used in this procedure, the chromatographic column was extensively washed with distilled water before shutdown. A secondary mobile phase, methanol, kept in the reservoir chamber of a programmable gradient unit (Du Pont 838) was also used to wash the chromatographic column before and after a series of analytical runs. Quantitation was obtained by measurement of peak heights, assuming proportionality between sample mass and peak height, as observed for Pth-amino acid standards.

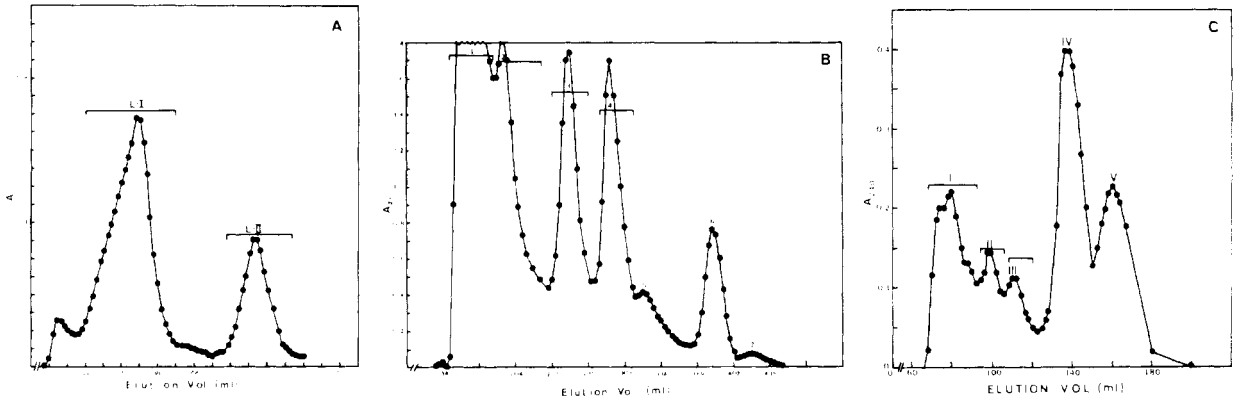

FIGURE 4: (A) Separation of CNBr digest of L-chain Hil. L chain (180 mg) was cleaved with CNBr (see text) and separated by gel filtration in a 5.0 × 90 cm column of Sephadex G-100, equilibrated with 1 M acetic acid and fitted with upflow adaptors. (B) Separation of tryptic digest of maleylated LI, CNBr fragment. Forty milligrams of a tryptic digest of maleylated LI fragment was loaded onto a 2.5 × 90 cm column of Sephadex G-50 superfine, equilibrated with 0.2 M ammonium bicarbonate and fitted with upflow adaptors. (C) Separation of a tryptic digest of demaleylated LII fragment (see Figures 3 and 5). About 10 mg of LII material was demalylated and digested with trypsin. The digest was loaded onto a 2.5 × 40 cm, Sephadex G-25 superfine column, equilibrated with 1 M acetic acid. Peaks IV and V were due to reagents and were not further processed.

TABLE I: Amino Acid Composition of L Chain Hil and Its CNBr Fragments.[a]

	L Chain		L-I		L-II	
Amino acid	Amino acid anal.[b]	Sequence	Amino acid anal.[c]	Sequence	Amino acid anal.[c]	Sequence
Asp	12.2	12	10.0	10	2.3	2
Thr	21.7	22	18.9	19	3.0	3
Ser[d]	29.3	29	23.6	24	5.4	5
Glu	24.0	24	18.0	18	6.0	6
Pro	17.4	17	10.8	11	5.8	6
Gly	11.9	12	10.1	10	2.2	2
Ala	19.8	20	14.9	15	5.0	5
½-cystine	5.0	5	nd	4	nd	1
Val[e]	15.0	15	11.7	12	2.9	3
Met	1.0	1	0.0	0	nd	1
Ile[e]	7.0	7	5.7	6	0.9	1
Leu	11.0	11	9.0	9	2.0	2
Tyr	10.7	11	7.4	7	3.7	4
Phe	4.8[f]	4	4.3	4	0.0	0
His	2.1	2	1.9	2	0.0	0
Lys	11.2	11	10.0	10	0.8	1
Trp	nd	4	nd	3	nd	1
Arg	4.9	5	3.2	3	1.7	2
Total		212		167		45

[a] Values are listed as moles per mole of L chain or fragment. [b] Average of nine analyses performed on aminoethylated L chain samples. Half-cystine has been calculated as cysteic acid on performic acid oxidized L chain samples. [c] Average of two analyses performed on aminoethylated samples. [d] Corrected for partial destruction during hydrolysis. [e] Corrected for incomplete hydrolysis. [f] High value presumably due to systematic error in automatic integration; the values for this residue in the tryptic peptides are in agreement with sequence data. In addition, no abnormal carry-over was observed in the steps following phenylalanine during automatic sequence determination. nd, not determined.

Amino acid analysis after HI hydrolysis (Smithies et al., 1971) was used to verify all sequence assignments, in particular toward the end of the automatic degradation runs, when background increased. The results obtained by this technique were not quantitated, since quantitation was already done by HPLC.

Fractionation and Purification of Peptides. The conditions for tryptic and chymotryptic digestions, separation and purification of peptides by gel filtration, paper electrophoresis and paper chromatography were as described before (Chen & Poljak, 1974). The overall scheme is illustrated in Figure 3. Cleavage by CNBr was performed as described (Gross, 1967). The products of the CNBr cleavage reaction were separated by gel filtration in 2.5 × 90 cm columns of Sephadex G-100 (Figure 4A) equilibrated with 1 M acetic acid and fitted with upflow adaptors. Peak LI (Figure 4A) was maleylated following a procedure described (Butler & Hartley, 1972) and digested with trypsin in 0.2 M NH_4HCO_3 buffer, pH 9.2. This tryptic digest was fractionated by gel filtration in a 2.5 × 90 cm column of Sephadex G-50 equilibrated with 0.2 M NH_4HCO_3, pH 9.2 (Figure 4B). Peak 1 from this separation (Figure 4B) was submitted to automated Edman degradation. Alternatively, samples of this material were demaleylated (Butler & Hartley, 1972) and digested with trypsin as above (Figure 4C). Other procedures and results obtained in the fractionation and purification of peptides are given in Figure 3. In addition to direct determination on Pth-amino acids, whenever possible amides were assigned on the basis of the electrophoretic mobility of peptides at pH 6.4.

Partial sequencing of tryptic peptides T2 and T8 by the dansyl-Edman procedure (Hartley, 1970) was performed as previously described (Chen & Poljak, 1974).

Amino Acid Analyses. They were carried out using a Durrum D-500 amino acid analyzer, equipped with a ninhydrin detection system.

Results

The amino acid compositions of L chain Hil and its CNBr fragments are shown in Table I. There is good agreement between the observed composition and that obtained from sequence analysis.

Two features of L chain Hil which are somewhat unusual in human immunoglobulin chains facilitated the sequence analysis reported in this communication. First, its α-amino terminal is unblocked, a feature which made possible automatic Edman degradation on the whole L chain. Second, a methionine residue at position 45 made it possible to obtain a fragment (LI, Figures 3 and 4A) which provided an internal starting point for automatic Edman degradation. Furthermore, a tryptic peptide spanning residues 61 to 189 could be obtained by digestion of maleylated LI material (1, Figure 4B). This peptide provided a second internal starting point for automatic Edman degradation. Thus, the whole V_L region was sequenced by automatic techniques (Figures 1 and 5).

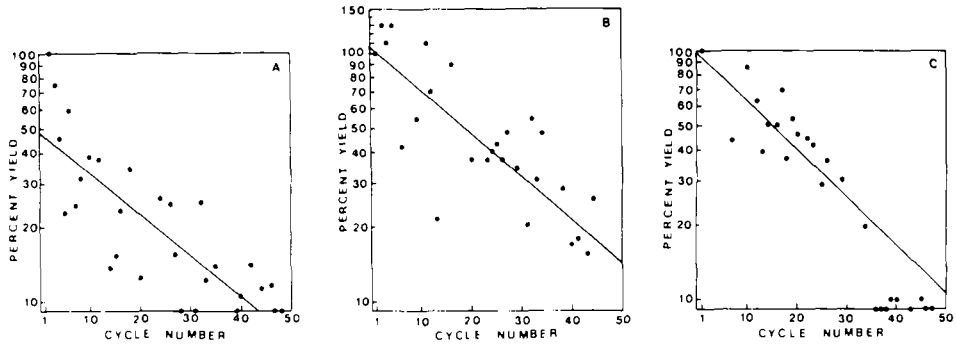

FIGURE 5: (A) Semilog plots of percent yields of Pth-amino acids from automatic degradation of L-chain Hil. Fifteen milligrams of L chain was sequentially degraded in an 890C Beckman sequencer using the protein quadrol program no. 122974. Quantitative yields of Pth-amino acids were obtained by HPLC as explained in the text. The average repetitive yield was 95.3%. The line shown in this figure was fitted to the points by a least-squares regression. (B) Semilog plot of percent yields of Pth-amino acids from an automatic degradation of the LI CNBr fragment (10 mg). Experimental conditions were as in Figure 5A. The average repetitive yield was 96%. (C) Semilog plot of percent yields of Pth-amino acids from an automatic degradation of the LII tryptic peptide (10 mg). Experimental conditions were as in Figure 5A. The average repetitive yield was 94%.

Three independent methods were used to identify every residue: HPLC; amino acid analysis after hydrolysis of the correspondent Pth with HI; and thin-layer chromatography. Wherever repetitive sequences were found, special consideration was given to carry-over ambiguities. The sequence Ser-Ser-Ser (62-64) presented no difficulty since it occurs near the beginning of the run of the maleylated L-I fragment, where the background and the carry-over were negligible. The sequence Gly-Gly-Gly (98-100) was unambiguously established after automatic sequencing and isolation and composition analysis of two overlapping peptides $T10_{C4}$ and $T10_{C5}$ corresponding to positions 98-102 and 98-103, respectively (Figure 1 and Table II).

Tryptic peptides and chymotryptic subpeptides covering the whole variable region were obtained and characterized (see Table II) to confirm the results obtained by automatic Edman degradation. The general strategy used in the purification of tryptic peptides is shown in Figure 3. Thus, the sequence of the V_L region can be considered as firmly established.

Information on the sequence of the constant C-terminal half, in particular at those positions (112, 114, 129, 143, 151, 156, 157, 163, 171, 186, and 189; see Fett & Deutsch, 1974; Lieu et al., 1977) at which amino acid replacements are known to occur was also obtained from tryptic peptides (Table II). This information is summarized in Figure 1 in which the tryptic peptides are ordered and assigned a sequence in agreement with their amino acid composition and with the established sequence for the C_L region of human chains. Positions 130-147 of the constant region were determined by automatic sequencing. A previously unobserved substitution was observed at position 155 to which an isoleucine residue is assigned instead of the usual valine. An attempt to confirm the occurrence of isoleucine at position 155 was made by digestion of tryptic peptide T13 (Figure 1), with a mixture of carboxypeptidases A and B. Only the C-terminal Lys residue was released. This negative result would be expected from the presence of a proline residue at position 154, N-terminal to isoleucine-155. Although the presence of isoleucine at position 155 was not ascertained by a direct sequencing step, compositional analysis of peptide T13 and sequence homology with other human λ chains makes the assignment of an isoleucine residue at position 155 reasonably clear.

Discussion

The study of the amino acid sequence of IgG Hil was undertaken in parallel with crystallographic studies of its Fab fragment. The sequence information presented in this communication will be essential for the complete interpretation of a high resolution Fourier map of Fab Hil, currently in preparation in this laboratory.

Comparison with the sequence of other human λ chains (see Kabat et al., 1976, for a comprehensive compilation) indicates that V_L Hil belongs to subgroup III. Positions 1, 8, 9, 11, 12, 14, 15, 20-22, 34, 39-41, 43, 45, 46, 53, 57, 58, 60-62, 64, 66, 67, 69, 72-75, 83, 85, 87, 97, 98, 100, 101, and 103-105 of V_L Hil are occupied by the same (thus far invariant) residues established in the sequence analysis of other subgroup III human λ chains. Residues 24, 63, and 70 of V_L Hil are different from those observed in other subgroup III human λ chains and in other human λ chains in general. However, the observed replacements, alanine for glycine at position 24, serine for glycine at position 63, and valine for alanine at position 70, are very conservative in terms of the possible mutational events that generated them (one base change in the genetic code). Inspection of the three-dimensional model of human λ chain New (Poljak et al., 1973, 1974) indicates that the replacements observed in V_L Hil at positions 24, 63, and 70 can be easily accommodated without distortions in the basic structure of "immunoglobulin-fold" of V_L.

It is of interest that the sequence of V_L Hil differs from that of V_L New by several features which should be related to the conformation of the combining sites of IgG New and IgG Hil. Thus, in the first hypervariable region V_L Hil lacks residues 27a, 27b, and 27c, a structural insertion which occurs in many human chains, including V_λ New. These residues are part of a short (one turn) helical region which constitutes one of the boundaries of the combining site in IgG New. Due to the fact that the second hypervariable region of V_L Hil (around position 50) is not followed by a deletion (so far unique to V_λ New), it is expected that the combining site will differ in this respect. Finally, the position occupied by Tyr-90 in V_λ New is replaced by Trp-90 in V_λ Hil. Since this residue contributes to an internal boundary of the combining site and is in close contact with H chain residues (Poljak et al., 1973, 1974), it is expected that the observed replacement will determine further structural differences between the combining sites of IgG Hil and IgG New.

The occurrence of a serine residue at position 152 and arginine at position 189 defines the isotype of Hil as Kern(−) (Hess et al., 1971), Oz(−) (Apella & Ein, 1967). Other substitutions that have been reported in the C_L region of human chains (Fett & Deutsch, 1974; Lieu et al., 1977), at positions

TABLE II: Amino Acid Composition of Tryptic Peptides of L Chain Hil.[a]

Amino acid[b]	T1	T2	T3	T4	T5	T6	T7	T8	T9	$T10_{C1}$	$T10_{C2}$
Asp			2.1 (2)	1.8 (2)	2.7 (2)				1.0 (1)		2.0 (2)
Thr	2.0 (2)	1.0 (1)			0.9 (1)				0.8 (1)	3.1 (4)	0.9 (1)
Ser	3.4 (4)[c]		1.0 (1)	0.9 (1)	1.2 (1)	0.3 (0)			1.1 (1)	3.2 (4)	1.0 (1)
Glu	2.8 (3)		1.2 (1)	1.2 (1)	1.1 (1)	2.0 (2)	1.8 (2)		2.0 (2)	0.3 (0)	3.0 (3)
Pro	2.9 (3)		1.0 (1)	0.9 (1)	0.9 (1)		1.0 (1)	1.0 (1)	1.8 (2)		
Gly	1.1 (1)				0.3 (0)	0.3 (0)			1.0 (1)	1.2 (1)	1.2 (1)
Ala	1.0 (1)		2.2 (2)	3.0 (3)	2.7 (3)			0.3 (1)[c]	0.4 (0)		2.1 (2)
Cys		(1)[e]			(1)[e]						
Val	1.8 (2)							1.0 (1)	0.9 (1)	0.8 (1)	1.2 (1)
Met[g]								(1)			
Ile		0.3 (1)[c]			0.7 (1)				1.3 (2)		0.9 (1)
Leu	1.0 (1)		1.0 (1)	1.1 (1)	1.1 (1)				0.4 (0)	1.0 (1)	
Tyr	1.1 (1)		1.1 (1)	1.7 (2)	2.3 (2)	1.2 (1)	1.1 (1)		1.0 (1)		1.7 (2)
Phe										1.0 (1)	
His											
Lys						0.8 (1)	0.8 (1)		0.8 (1)		
Trp[d]						(1)	(1)				
Arg	0.8 (1)						1.1 (1)		1.8 (2)		
Total	19	3	9	11	14	15	8	4	15	12	14
% yield[h]	15	11	7	13	4	4	18	7	100	18	15
Residue no.	1–19	20–22	23–31	23–33	20–33	34–38	34–41	42–45	46–60	61–72	73–86

	$T10_{C3}$	$T10_{C4}$	$T10_{C5}$	T11	T12	$T10_{C6}$	T13	T14	T15	T16	T17
Asp	2.0 (2)				1.0 (1)	1.2 (1)	0.9 (1)		1.9 (2)	2.3 (2)	
Thr		0.9 (1)	1.0 (1)	1.0 (1)	1.1 (1)	1.0 (1)		2.8 (3)	1.1 (1)	1.3 (1)	3.7 (4)
Ser	2.0 (2)				3.2 (3)	0.3 (0)	2.1 (2)	1.1 (1)	3.8 (4)	3.9 (5)	4.0 (4)
Glu	1.2 (1)			1.1 (1)	3.1 (3)	0.9 (1)		1.1 (1)	3.0 (3)	2.8 (3)	4.3 (4)
Pro				0.9 (1)	3.0 (3)		1.0 (1)	0.9 (1)	0.9 (1)	1.1 (1)	1.0 (1)
Gly	1.2 (0)	2.8 (3)	3.0 (3)	1.1 (1)		0.4 (0)		1.1 (1)	0.5 (0)	0.6 (0)	1.2 (1)
Ala	2.0 (2)				2.8 (3)	1.9 (2)	1.0 (1)	0.7 (1)[c]	2.0 (2)	2.1 (2)	1.2 (1)
Cys	0.8 (1)[f]										2.0 (2)[f]
Val				1.0 (1)	0.9 (1)			1.0 (1)	0.3 (0)	0.6 (0)	3.0 (3)
Met[g]											
Ile	1.0 (1)						1.1 (1)			0.5 (0)	
Leu			1.0 (1)	2.0 (2)	1.9 (2)	1.0 (1)			2.0 (2)	2.1 (2)	
Tyr									1.9 (2)	1.8 (2)	1.1 (1)
Phe	0.9 (1)				1.1 (1)					0.3 (0)	
His				0.9 (1)						0.7 (1)	0.9 (1)
Lys		1.1 (1)	0.8 (1)		0.9 (1)	0.9 (1)	1.0 (1)	0.7 (1)	1.9 (2)	2.3 (2)	0.9 (1)
Trp[d]	nd (1)								nd (1)	nd (1)	
Arg									0.3 (0)	0.7 (1)	
Total	11	5	6	8	19	7	7	10	20	23	23
% yield[h]	9	11	18	13	50	4	9	18	2	17	82
Residue no.	87–97	98–102	98–103	103–110	111–129	126–132	150–156	157–166	167–186	167–189	190–212

[a] Values listed as residues/mol. Values smaller than 0.3 are not given. Numbers in parentheses are the assumed integral values. [b] Experimental values not corrected for destruction, except for serine, where a recovery of 85% has been assumed. [c] N-terminal residue, partially destroyed by reaction with ninhydrin. [d] Analyzed qualitatively. [e] Analyzed qualitatively as aminoethylcysteine. [f] Analyzed as carboxymethylcysteine. [g] Analyzed qualitatively as homoserine. [h] Estimated from amount of material digested. Values are normalized to a maximum recovery of 50% for peptides obtained from paper techniques. nd, not determined.

112, 114, 129, 143, 151, 156, 157, 163, 171, and 186 are not observed in L chain Hil which conforms to the most commonly observed C_λ sequences. However, a substitution which has so far not been detected in other human λ chains occurs at position 155, in which an isoleucine residue replaces the commonly found valine. Since our data are limited to L chain Hil, it cannot be ascertained whether or not this is a frequently occurring substitution in normal human λ chains. Such a substitution could be coded by a λ chain gene or a λ chain allele different from those detected and reported before. Obviously, the detection of such a substitution requires special attention such as the isolation and amino acid analysis of tryptic peptide T13 (Figure 1), since a replacement of valine by isoleucine may not be detected by the electrophoretic and/or chromatographic properties of that peptide. In addition, this replacement will not be detected serologically on native L chains since the amino acid chain is not exposed to solvent in the three-dimensional structure. However, the fact that it has not been detected before by sequence analysis can be taken as a tentative indication that it is not the product of a frequently expressed gene or allele in human populations. The substitution of valine (155) by isoleucine reported here brings to 13 the number of positions at which replacements have thus far been found in the C region of human λ chains. These (and other possible undetected substitutions) suggest that human λ chains exhibit considerable complexity at the genetic level.

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Isolation, Purification, and Properties of Mouse Heavy-Chain Immunoglobulin mRNAs†

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ABSTRACT: A procedure is described for the isolation of highly purified heavy-chain immunoglobulin mRNAs from a variety of mouse plasmacytomas (IgA, IgG, and IgM producers). The use of fresh tissue and the rapid isolation and direct extraction of membrane-bound polyribosomes were found to be essential in obtaining large quantities of undegraded heavy-chain mRNAs. The individual mRNAs were purified by two cycles of oligo(dT)-cellulose chromatography, sodium dodecyl sulfate-sucrose gradient centrifugation, and electrophoresis on 98% formamide containing polyacrylamide gels. When added to a cell-free protein-synthesizing system from wheat germ, the MPC-11 γ_{2b} and H2020 α heavy-chain mRNAs efficiently directed the synthesis of a predominant product of 55 000 molecular weight, while the synthesis of a 70 000 dalton protein in addition to other lower molecular weight polypeptides were observed with MOPC 3741 μ mRNA. All of these proteins were immunoprecipitable with class-specific heavy-chain antisera, and in the case of the γ_{2b} in vitro products good correspondence in a comparative trypsin-chymotrypsin fingerprint with in vivo labeled γ_{2b} heavy chain was observed. The γ_{2b} and α heavy-chain mRNAs possessed a chain length of ~1800 nucleotides and the μ mRNA a size of ~2150 nucleotides when examined under stringent denaturation conditions. The purities of the α, γ_{2b}, and μ mRNAs were estimated to be 60-80%, 50-70%, and 50-83%, respectively, on the basis of their hybridization rates with cDNA probes in comparison to mRNA standards of known complexity. Heavy-chain mRNAs of the same class isolated from different mouse strains (Balb/C or NZB) display no detectable sequence differences in cross hybridization experiments, even though the cDNA-mRNA hybrids are submitted to stringent S_1 nuclease digestion. These results indicate that allotypic determinants represent only a minor fraction of the heavy-chain constant region sequence in the mouse.

In order to understand the complex mechanisms responsible for the diversification and translocation of immunoglobulin (Ig) genes and for the subsequent accumulation of the Ig mRNAs which direct the synthesis of the heavy- and light-chain polypeptides, procedures for the isolation of highly purified, biologically active heavy- and light-chain mRNAs are essential. Over the past 6 years, κ and λ light-chain mRNAs have been purified, structurally characterized, and used for studies of the repertoire of mouse light-chain genes by nucleic acid hybridization techniques (see review by Kuehl, 1977) and more recently for an investigation of the mechanisms of Ig light-chain gene translocation (Hozumi and Tonegawa, 1976; Tonegawa et al., 1976, 1977).

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