The Covalent Structure of a Human γG-Immunoglobulin. V. Partial Amino Acid Sequence of the Light Chain*

Bruce A. Cunningham,† Paul D. Gottlieb, William H. Konigsberg, and Gerald M. Edelman

ABSTRACT: The amino acid sequence of the variable region of a κ chain isolated from a γG myeloma protein (Eu) has been determined. In addition, the tryptic peptides of the constant portion have been isolated and partially sequenced. Comparison with Bence–Jones proteins of the same antigenic class and subgroup showed that the variable portion of Eu has 13 new substitutions at positions which have not previously been observed to vary. Seven additional new interchanges were found at positions known to be variable in κ chains. Five of the twenty new amino acid interchanges require a two-base change in the codon specifying amino acids at that position.

The data on the constant region suggest that it has the same sequence as that of Inv 3 κ chains with one exception. The residue at position 108 of the light chain is glycine rather than arginine. Thus this position may be in the variable region or it may represent a site for allotypic variation in the constant region. In spite of the high degree of variability in the sequence of the Eu κ chain as compared with the sequences of Bence–Jones proteins, the extent of homology is consistent with the previous conclusion that urinary Bence–Jones proteins are light chains.

Analysis of the primary structure of immunoglobulins was facilitated by the demonstration that urinary Bence–Jones proteins are light chains (Edelman and Gally, 1962; Schwartz and Edelman, 1963) similar to those found in serum myeloma proteins and antibodies. Because of the availability, homogeneity, and variety of Bence–Jones proteins, their amino acid sequences have been studied in a number of laboratories (Hilschmann and Craig, 1965; Titani *et al.*, 1967; Milstein, 1966; Gray *et al.*, 1967; Hilschmann, 1967). These studies indicate that Bence–Jones proteins consist of a variable region (residues 1–107) and a constant region (residues 108–214). In Bence–Jones proteins of antigenic type K, differences are observed in only one position of the constant region, and these reflect allotypic variation (Baglioni *et al.*, 1966). In contrast, a comparison of the variable regions of different Bence–Jones proteins of the same antigenic class shows that amino acid interchanges occur in as many as 60 positions in κ chains.

It is currently believed that the diversity of amino acid sequence is related to the diverse specificities of antibodies (Edelman and Gally, 1967) and that a similar structural pattern will emerge from studies of the primary structure of heavy chains. The determination of the primary structure of a myeloma protein provides an opportunity to compare the amino acid sequence of light and heavy chains from the same molecule. The primary structure of the light chain isolated from a serum myeloma protein can also be compared with the known amino acid sequences of various urinary Bence–Jones proteins.

In the present paper, the amino acid sequence of the variable region of a κ chain from the myeloma protein Eu and the composition and partial sequence of peptides from the constant region are compared with amino acid sequences of Bence–Jones proteins of the same antigenic class.

Materials and Methods

Preparation of Light Chains and Fab(t).[1] The procedures employed for the isolation of light chain and the enzymatic fragment Fab(t) from the myeloma protein Eu are described in the first paper in this series (Edelman *et al.*, 1968). Prior to any further treatment, light-chain preparations were filtered on columns of Sephadex G-100 in 1 M propionic acid. For 100–300 mg of light chains, column dimensions were 2.5 × 200 cm. Less than 5% of the protein emerged in the void volume, and the remainder emerged from the column as a single peak in the expected position.

Preparation of CNBr Fragments of the Light Chain.

* From The Rockefeller University, New York, New York, and the Department of Biochemistry, Yale University, New Haven, Connecticut (W. H. K.). *Received January 8, 1968.* This work was supported in part by grants from the National Science Foundation (GB-6546 and GB-6655) and the U. S. Public Health Service (AM 04256-08 and GM 12607-03). Part of this work was presented at the 32nd Cold Spring Harbor Symposium on Quantitative Biology, 1967 (Waxdal *et al.*, 1967), the Nobel Symposium 3 on γ-Globulins, Södergarn, Sweden, 1967 (Edelman, 1967), and the 7th International Congress of Biochemistry, Tokyo, 1967 (Edelman *et al.*, 1967). The nomenclature used is that recommended by the World Health Organization (1964).

† Postdoctoral fellow of the National Science Foundation.

[1] Abbreviations used that are not listed in *Biochemistry 5* 1445 (1966), are: Fab(t), tryptic fragment corresponding to Fab (World Health Organization, 1964); dansyl, 1-dimethylaminonaphthalene-5-sulfonyl; Asx, aspartic acid or asparagine; Glx, glutamic acid or glutamine.

The preparation of CNBr fragments of the light chain has been described (Waxdal *et al.*, 1968b).

Preparation of Tryptic Peptides. Following complete reduction of 827 mg of Eu light chain and alkylation with iodoacetamide (Waxdal *et al.*, 1968b), the dialyzed solution (90 ml, pH 4.3) was adjusted to pH 8.0 with 0.8 N ammonium hydroxide and 8 mg of trypsin (Worthington Biochemical Corp., Freehold, N. J.; two-times crystallized, lot no. 6223) was added. Digestion was allowed to proceed at room temperature, and the solution was maintained at pH 8.0 by the addition of 0.8 N ammonium hydroxide with an Agla micrometer syringe and Radiometer TTT1b pH-Stat. After 3 hr, an additional 8 mg of trypsin was added. After an additional 7.5 hr, the suspension was brought to pH 3.0 with 88–90% formic acid. The suspension was centrifuged to remove the insoluble material, and the clear supernatant solution which contained only about 50% of the total digest was lyophilized.

For the tryptic digestion of CNBr fragments L_1 and L_2, 100–220 mg of a mixture of these fragments was suspended in 20 ml of distilled water and sufficient 1 N ammonium hydroxide was added to adjust the pH to 7.8 or 8.0. Trypsin (1.0–2.2 mg, 1% by weight of the CNBr fragments) (Calbiochem, Los Angeles, Calif., lot no. 65345), treated with L-1-tosylamino-2-phenylethyl chloromethyl ketone, was added, and the suspension was maintained at 37° and pH 7.8 or 8.0 by addition of 0.5–1.0 N ammonium hydroxide. After 4 hr, the suspension was diluted with water and lyophilized.

Preparation of Chymotryptic Peptides. The procedure for the chymotryptic digestion of 60 mg of a mixture of CNBr fragments L_1 and L_2 and 90 mg of CNBr fragment L_3 was identical with that used for tryptic digestion of L_1 and L_2 except that 1 mg of α-chymotrypsin (Worthington Biochemical Corp., Freehold, N. J.; three-times crystallized, CD1-6150-1) was used in place of trypsin.

Tryptic peptides LT3 and LT7 were also digested with chymotrypsin. To a suspension of 1.1 μmoles of LT3 in 5 ml of 0.05 M Tris-HCl (pH 8.0) was added 50 μl of a 0.1% solution of α-chymotrypsin. After 3 hr at 37° an additional 50 μl of enzyme solution was added, and after 19 hr at 37° the clear solution was lyophilized. To a solution of 3 μmoles of LT7 in 20 ml of distilled water at 37° and pH 8.0 was added 0.1 mg of α-chymotrypsin, and the pH was maintained in the pH-Stat by the addition of 0.1 N ammonium hydroxide. After 7 hr the reaction mixture was lyophilized.

Digestion with Carboxypeptidases A and B. The determination of carboxyl-terminal residues by digestion with carboxypeptidases A and B was carried out as described by Weber and Konigsberg (1967) except that 0.02 M sodium barbital (pH 7.6) was used.

Ion-Exchange Chromatography. Mixtures of peptides were chromatographed on Bio-Rad AG50WX4 (30–35 μ) (Bio-Rad Laboratories, Richmond, Calif., lot no. 3934) in jacketed columns maintained at 38°. For 8–20 μmoles of peptide, column dimensions were 0.9 × 40 cm; for 1–10 μmoles of peptide, column dimensions were 0.9 × 15 cm. A linear gradient was employed ranging from 0.2 M pyridine brought to pH 3.1 with acetic acid to 2.0 M pyridine adjusted to pH 5.5 with acetic acid. For the smaller columns, 150–200 ml of each buffer was used; for the larger column, the volume was increased to 350 ml. Spectral grade pyridine (Mallinckrodt Chemical Works, St. Louis, Mo.) was used to prepare all buffers. Flow rates were maintained at 30 ml/hr by means of a miniPump (Milton Roy Co., Philadelphia, Pa.) and fractions of 1–2 ml were collected.

Peptides were also fractionated on Bio-Rad AG1X4 (−400 mesh, lot no. 2943) according to the method of Schroeder and Robberson (1965) in jacketed columns at 38° or at room temperature. Gel filtration was carried out using Sephadex G-50 fine, G-25 fine, or G-10 fine (Pharmacia, Uppsala, Sweden).

Analysis of Effluent Fractions. The ultraviolet absorbance at 280 and 230 mμ of the effluent fractions from Sephadex columns was measured with a Zeiss PMQII spectrophotometer. Ninhydrin analysis of the effluent fractions from some Sephadex columns and all ion-exchange columns was carried out manually (Moore and Stein, 1954) or automatically using an AutoAnalyzer (Technicon Corp., Ardsley, N. Y.) equipped with an automatic sampler attachment fitted to a Research Specialties Co. (Richmond, Calif.) Model 1205A fraction collector. In the latter case, the signal from the colorimeter served as a single input to a linear recorder. Consequently the observed signal was proportional to transmittance rather than absorbance. An arbitrary scale of 0–3.0 was adopted, and all data from this instrument are reported as A_{570}*. The effluent from the ion-exchange column used to fractionate tryptic peptides from the whole light chain was monitored continuously as described previously (Waxdal *et al.*, 1968a).

Amino acid analysis of peptides was carried out as described previously (Edelman *et al.*, 1968). Hydrolyses were carried out for 18–22 hr at 110°.

Amino-Terminal Analysis. Amino-terminal residues of peptides were identified as the dansyl derivatives. Aliquots for dansylation (5–10 nmoles of peptide) were transferred to small Pyrex test tubes (10 × 75 mm) and dried. Samples were dansylated as described by Gray (1967a). Following dansylation, the dried samples were dissolved in 200 μl of 6 N HCl, evacuated for 5 min, and hydrolyzed for 18 hr at 110°.

Large, insoluble fragments and whole proteins were dansylated in urea as described by Gray (1967a). When the protein precipitated during dansylation, it was centrifuged and washed three times with distilled water. HCl (500 μl of 6 N) was added, the tube was evacuated for 15 min, and the sample was hydrolyzed for 18 hr at 110°.

Dansylamino acids were identified by two-dimensional thin-layer chromatography on polyamide layers (Cheng Chin Trading Co., Hankow St., Taipei, Taiwan) using solvent 1 (200 ml of water and 3 ml of 90% formic acid) and solvent 3 (60 ml of *n*-heptane, 60 ml of *n*-butyl alcohol and, 20 ml of glacial acetic acid) of Woods and Wang (1967) for the first and second dimensions, respectively. All samples were spotted in acetone and were dried under a stream of air. Standards (Calbiochem, Los Angeles, Calif.) were simultaneously chromatographed in the margins on the same sheet as the sample,

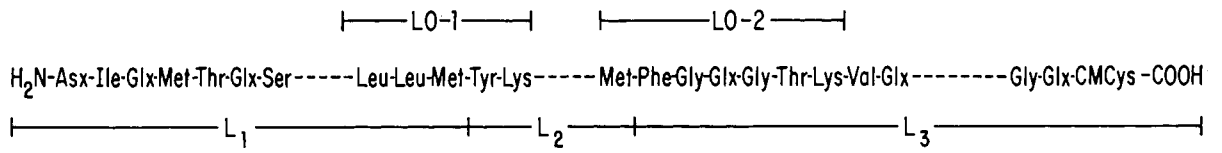

FIGURE 1: Partial structure of the Eu light chain based on sequence analysis of the intact light chain, of fragment L_3, and of tryptic peptides LO-1 and LO-2. The COOH-terminal tripeptide Gly-Glx-CMCys (see Table IX, peptide LT20) was isolated from a tryptic digest of the light chain.

and highly resolved chromatograms could be obtained with up to seven standards per chromatogram. Ammonia was never completely eliminated from the samples, and the dansylamide served as an excellent internal standard. Fluorescent spots were located by means of a shortwave ultraviolet lamp (Mineralight UVS-11, Ultraviolet Products, Inc., San Gabriel, Calif.).

Determination of Amino Acid Sequence. The amino acid sequence of peptides was determined qualitatively by a combination of the Edman degradation (Edman, 1950) and the dansyl end-group techniques described above. The Edman degradation was performed as described by Gray (1967b). All solvents and reagents were purified and redistilled. Following each step of the Edman degradation, the newly exposed amino-terminal residue was determined as the dansyl derivative. Although the qualitative method was used routinely, aliquots were taken for subtractive Edman analysis whenever sufficient peptide was available. Aliquots for subtractive Edman analysis (25–35 nmoles of peptide) were transferred to small Pyrex test tubes (10 × 75 mm) and dried *in vacuo* over concentrated H_2SO_4 and NaOH pellets. The dried samples were then dissolved in 0.3 ml of glass-distilled water and extracted three times with 1-ml portions of *n*-butyl acetate. The aqueous phase containing peptide was dried *in vacuo*. About 200 μl of 6 N HCl was added, the tubes were evacuated for 20 min and the samples were hydrolyzed for 22 hr at 110°. After hydrolysis, samples were dried *in vacuo* over NaOH pellets and stored at −20° until needed to check any questionable results obtained by the dansyl method.

Proline and tryptophan residues presented special problems. Dansylproline does not survive 18-hr hydrolysis at 110°, and whenever proline was suspected, a 6-hr hydrolysis was performed. If an 18-hr hydrolysate failed to reveal a dansylamino acid upon chromatography, the subtractive Edman sample was used for identification.

When tryptophan was the amino-terminal residue, dansylation and acid hydrolysis failed to yield dansyltryptophan upon chromatography. The presence of tryptophan in the original peptide was detected spectrophotometrically or by staining on paper with Ehrlich's reagent (Block *et al.*, 1955). When a peptide was known to contain tryptophan and no dansyl end group or loss of any other amino acid upon subtractive Edman analysis was observed, the presence of tryptophan at that position was assumed. This was usually confirmed by isolation of the tryptophan-containing chymotryptic peptide from the original tryptic peptide and the determination of tryptophan by treatment with carboxypeptidase A and amino acid analysis.

When lysine was the amino-terminal residue in the original peptide, the dansyl procedure yielded α,ε-didansyllysine on chromatography. After several Edman degradations, the ε-amino group of any lysine still present appeared to be blocked by a phenylthiocarbamyl group. Dansylation of a peptide containing ε-phenylthiocarbamyllysine as its amino-terminal residue yielded a faint but definite spot upon chromatography, but no α,ε-didansyllysine. This spot was assumed to be α-dansyl-ε-phenylthiocarbamyllysine, and was used for the provisional identification of lysine until subtractive Edman analysis could be performed. In general, when a tryptic peptide containing one lysine or one arginine residue was sequenced, the carboxyl-terminal amino acid was assumed to be the basic residue.

Dansylisoleucine and dansylleucine are somewhat difficult to resolve by chromatography, and whenever possible the subtractive Edman technique was used to determine these residues. In the case of a large peptide or fragment where the subtractive Edman procedure could not be used, definitive identification of a leucine or isoleucine was made only after isolation and sequencing of a smaller peptide containing this residue.

Results

Previous studies (Waxdal *et al.*, 1968b) have shown that the order of the CNBr fragments of the light chain is L_1–L_2–L_3 and have established the amino acid sequence around each of the points of cleavage by CNBr. The partial structure of the light chain based on these data and on sequence analysis of the intact light chain and the CNBr fragments is summarized in Figure 1. The amino acid composition and molecular weight of the light chain have been reported by Edelman *et al.* (1968).

Sequence analysis of the intact light chain by the dansyl-Edman procedure gave residues 1–7 of L_1. The peptide Leu-Leu-Hsr was isolated from a tryptic digest of a mixture of L_1 and L_2. From information on the overlap peptide LO-1 (Waxdal *et al.*, 1968b) we placed this tripeptide at the carboxyl terminus of L_1. The dipeptide Tyr-Lys was isolated from the same tryptic digest. The observed amino-terminal residues of L_1 and L_2 mixtures, aspartic acid or asparagine and tyrosine, indicated that Tyr-Lys was the amino-terminal tryptic peptide of L_2. The sequence of the first eight residues of L_3 was also determined by the dansyl-Edman procedure. The first six residues corresponded to the last six residues of overlap peptide LO-2 (Waxdal *et al.*, 1968b). The tripeptide Gly-Glx-CMCys was isolated from a tryptic digest of the whole light chain and was tentatively placed at the carboxyl terminus of L_3.

TABLE I: Amino Acid Composition of Tryptic Peptides from L_1 and L_2 Mixtures.[a]

	LT-1′	LT1B	LT-2	LT-3	LT-4	LT-5A	LT-5B	LT-6	LT-7	LT-8A
Lys				2.0 (2)	1.1 (1)		1.0 (1)		0.9 (1)	
Arg	1.0 (1)	0.9 (1)	1.0 (1)					1.0 (1)	0.3	
Hsr[b]	1.0 (1)					0.6 (1)				1.0 (1)
CMCys			0.8 (1)						0.8 (1)	
Asp	2.0 (2)	1.0 (1)		1.1 (1)					3.6 (4)	
Thr	1.9 (2)	1.9 (2)	1.8 (2)	1.1 (1)					4.1 (4)	
Ser	3.5 (4)	3.3 (4)		2.1 (2)				3.5 (4)	5.5 (6)	
Glu	2.0 (2)	1.1 (1)		2.9 (3)	0.3			1.1 (1)	4.3 (4)	
Pro	1.0 (1)	1.0 (1)		1.1 (1)	0.9 (1)			1.1 (1)	1.4 (1)	
Gly	1.0 (1)	1.1 (1)		1.1 (1)	0.3			1.0 (1)	3.1 (3)	
Ala	1.0 (1)	0.8 (1)		1.7 (2)	0.9 (1)			0.9 (1)	1.4 (1)	
Val	1.0 (1)	0.9 (1)	1.0 (1)					1.0 (1)	0.4	
Ile	0.9 (1)		0.9 (1)	1.0 (1)					2.1 (2)	
Leu	0.9 (1)	1.0 (1)		1.0 (1)		2.0 (2)		1.0 (1)	2.2 (2)	
Tyr				1.3 (1)			0.8 (1)		2.4 (3)	
Phe									2.9 (3)	
Trp				(2)						
Total residues	18	14	6	18	3	3	2	11	35	1
Yield[c] (%)	14	11	42	15	41	42	19	14	45	20

[a] Values reported are amino acid residues. Amino acids present at a level less than 0.2 residue are omitted. Values in parentheses represent nearest integral number of residues. [b] Includes homoserine and homoserine lactone. [c] Yields are based on micromoles of peptides isolated compared with micromoles of protein originally digested with trypsin.

Because of the importance of the variable region of the light chain, efforts were concentrated on determining the amino acid sequence of CNBr fragments L_1 and L_2 and the amino-terminal portion of L_3. The elucidation of the amino acid sequences of L_1 and L_2 is presented below, followed by the determination of the sequence of the amino-terminal residues of L_3. In addition, preliminary evidence for the amino acid sequence of the remainder of L_3 is presented.

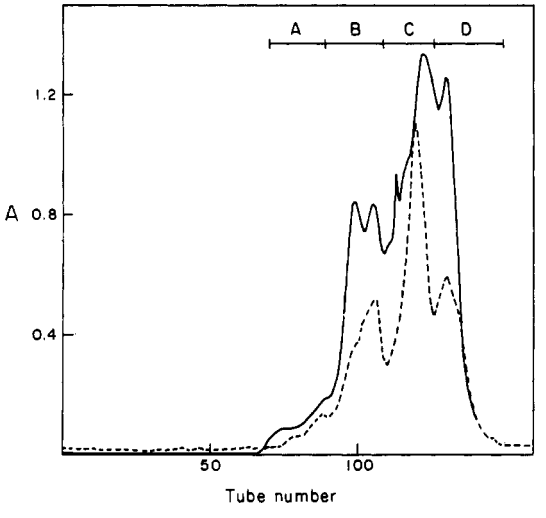

FIGURE 2: Gel filtration of tryptic peptides of L_1 and L_2 soluble in 1 M propionic acid on a column (2.1 × 100 cm) of Sephadex G-25 in the same solvent. The absorbance at 280 mμ of the effluent fractions is indicated by the solid line. The absorbance at 570 mμ of 0.1-ml aliquots from alternate tubes after ninhydrin analysis is represented by the dashed line. Each tube contained 2.2 ml of the effluent.

Peptides were isolated from tryptic digests of mixtures of CNBr fragments L_1 and L_2 and the amino acid sequence of each peptide was determined. The order of the tryptic peptides in these CNBr fragments was determined both by comparison of the amino acid sequence of the tryptic peptides with the partial structure of the light chain (Figure 1) and by the isolation and characterization of peptides prepared by chymotryptic digestion of mixtures of L_1 and L_2.

Isolation of Tryptic Peptides from Mixtures of L_1 and L_2. The amino acid compositions of the peptides isolated from tryptic digests of mixtures of L_1 and L_2 are summarized in Table I. Tryptic peptides obtained from a mixture of L_1 and L_2 prepared by CNBr cleavage of Fab(t) were extracted twice with 1 M propionic acid. The supernatant fraction containing 74% of the digest was filtered on a column of Sephadex G-25 (Figure 2). Fraction A contained a small amount of a mixture of larger peptides which was not resolved. Fractions B–D contained mixtures of peptides which were further fractionated by ion-exchange chromatography on AG1X4. A mixture of peptide LT1B and LT6 (Table I) was obtained from fraction B and was resolved by ion-exchange chromatography on AG50X4. Fraction C

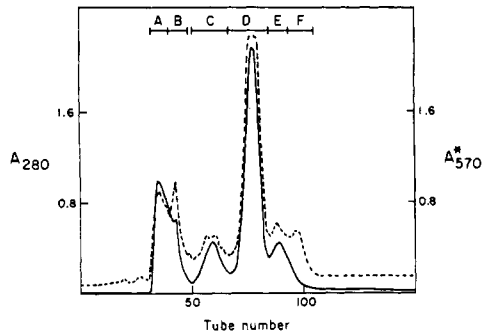

FIGURE 3: Fractionation of tryptic peptides of L_1 and L_2 insoluble in 1 M acetic acid on a column (2.5 × 100 cm) of Sephadex G-50 in 5% butyl alcohol–2 M acetic acid. The absorbance at 280 mμ of effluent fractions is indicated by the solid line (left ordinate). The ninhydrin color yield (A_{570}*) was determined automatically as described in Materials and Methods and is represented by the dashed light (right ordinate). Each tube contained 3.2 ml.

yielded peptides LT2 and LT4. Peptide LT5A was also obtained from fraction C but required further purification by ion-exchange chromatography on AG50X4. Peptides LT5B and LT8A were obtained from fraction D.

Gel filtration of the tryptic peptides soluble in 1 M acetic acid obtained from a digest of L_1 and L_2 isolated from the light chain gave an elution pattern similar to that shown in Figure 2. Peptide LT1′ (Table I) was located in the region corresponding to fractions A and B in Figure 2 and was purified by gel filtration on Sephadex G-50 followed by ion-exchange chromatography on AG50X4.

In every tryptic digest of mixtures of L_1 and L_2, 25–35% by weight of the digested material was insoluble in 1 M acetic acid or 1 M propionic acid. In order to isolate peptides from this insoluble material, gel filtration in *n*-butyl alcohol–acetic acid–water or *n*-propyl alcohol–acetic acid–water mixtures was employed. That portion of a tryptic digest of a mixture of L_1 and L_2 which was insoluble in 1 M acetic acid was dissolved in 5% butyl alcohol–2 M acetic acid and filtered on Sephadex G-50 in the same solvent (Figure 3). Fractions A and B had amino acid compositions similar to LT7 (Table I) but did not give integral values of amino acid residues. Fraction C contained a mixture of peptides which was not fractionated further. Fraction D was filtered on the same column to yield LT3. Fraction E contained a small amount of a mixture of peptides, and fraction F contained no amino acids or peptides.

TABLE II: Amino Acid Sequence of Tryptic Peptides from Mixtures of L_1 and L_2.

Eu L	Asx-Ile-Glx-Met-Thr-Glx-Ser-
Lt 1′	Asx-Ile-Glx-Hsr(Thr,Glx,Ser,Pro,Ser,Thr,-Leu,Ser,Ala,Ser,Val,Gly,Asx)Arg
LT 1B	Thr-Glx-Ser-Pro-Ser-Thr-Leu-Ser-Ala-Ser-Val-Gly-Asx-Arg
LT 2	Val-Thr-Ile-Thr-CMCys-Arg
LT 3	Ala-Ser-Glx-Ser-Ile-Asx-Thr(Trp)Leu-Ala-(Trp)Tyr(Glx,Glx,Lys,Pro,Gly)Lys
LT 4	Ala-Pro-Lys
LT 5A	Leu-Leu-Hsr
LT 5B	Tyr-Lys
LT 6	Ala-Ser-Ser-Leu-Glx-Ser-Gly-Val-Pro-Ser-Arg
LT 7	Phe-Ile-Gly-Ser-Gly-Ser-Gly-Thr-Glx-Phe-Thr-Leu(Thr,Ile,Ser,Ser,Leu,Glx,Pro,Asx,-Asx,Phe,Ala,Thr,Tyr,Tyr,CMCys,Glx,Glx,-Tyr,Asx,Ser,Asx,Ser)Lys
LT 8A	Hsr

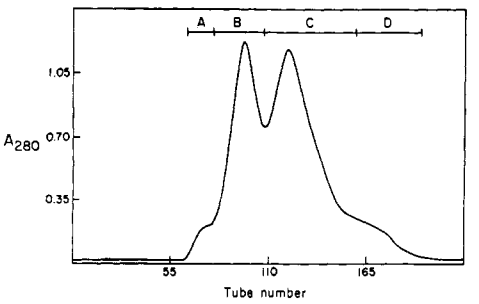

FIGURE 4: Gel filtration of a tryptic digest of L_1 and L_2 on a column (2.5 × 90 cm) of Sephadex G-50 in *n*-propyl alcohol–acetic acid–water (1:1:3, v/v). The absorbance of the effluent fractions at 280 mμ is represented by the solid line. Each tube contained 2.0 ml of effluent.

Gel filtration of a tryptic digest of a mixture of L_1 and L_2 on Sephadex G-50 in *n*-propyl alcohol–acetic acid–water (1:1:3, v/v), a solvent in which the entire tryptic digest was soluble, yielded two major fractions (Figure 4). After lyophilization, the peptide mixture obtained from fraction B was extracted with 2% acetic acid. The remaining solid was dissolved in the propyl alcohol–acetic acid–water mixture and filtered on Sephadex G-50 to yield peptide LT7 (Table I). Further attempts to purify this peptide by ion-exchange chromatography on AG1 (Hilschmann and Craig, 1965) were unsuccessful.

Amino Acid Sequence of Tryptic Peptides of L_1 and L_2. Tryptic peptides LT1B, LT2, LT4, LT5A, LT5B, and LT6 were sequenced, and the results are presented in Table II. Because of their large size, LT1′, LT3, and LT7 could not be sequenced entirely (Table II). The determination of the complete amino acid sequence of these peptides is described below. The sequence of the first seven residues of the light chain is included in Table II for purposes of comparison.

The complete sequence of LT1′ was deduced from the sequence of the first four residues of this peptide, the sequence of the first seven residues of the light chain, and the complete sequence of LT1B. Since LT1′ and LT1B were isolated from CNBr fragments of the light chain, we conclude that the absence of residues 1–4 in LT1B is the result of CNBr cleavage at the methionine residue at position 4 of the light chain and that the presence of homoserine in position 4 of LT1′ is the result of a modification of this methionine. A peptide has been isolated from a tryptic digest of light chains (*cf.* Table IX) which has the same composition as LT1′ except that it contains a residue of methionine and no homo-

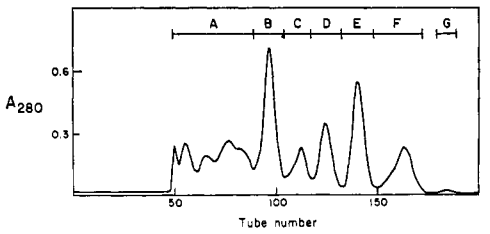

FIGURE 5: Initial fractionation of the chymotryptic peptides of L_1 and L_2 by gel filtration on a column (2.5 × 100 cm) of Sephadex G-25 in 5% formic acid. Each tube represents 2.9 ml of effluent, and the solid line indicates the absorbance at 280 mμ.

serine. This peptide begins with the sequence Asx-Ile-Glx-Met. From this data and the data in Table II, the sequence of the amino-terminal 18 residues of Eu light chain is: Asx-Ile-Glx-Met-Thr-Glx-Ser-Pro-Ser-Thr-Leu-Ser-Ala-Ser-Val-Gly-Asx-Arg-.

The complete sequence of LT3 was determined from the partial sequence presented in Table II and from sequence analysis of chymotryptic peptides (Table III) derived from LT3 and mixtures of L_1 and L_2. The peptides LT3C1, LT3C2, LT3C3, and LT3C4 were isolated by gel filtration of a chymotryptic digest of LT3 on Sephadex G-25 in 2% acetic acid. The sequence of LT3 is given in Table IV. The presence of tryptophan residues at positions 8 and 11 and tyrosine at position 12 was established by treatment of LT3C1, LT3C2, and LT3C3, respectively, with carboxypeptidase A. Peptide LT3C4 (Table III) was not sufficiently pure for accurate sequence analysis. Peptide LC6 (*cf.* Table VII) was isolated from a chymotryptic digest of L_1 and L_2, and the amino-terminal sequence was determined as Tyr-Glx-Glx-Lys-(Pro)-Gly by the dansyl-Edman procedure. Verification of the sequence by the subtractive Edman technique was hampered by the failure to remove quantitatively the glutamine (or glutamic acid) residues, probably as the result of pyrrolidonecarboxylic acid formation. However, a definite decrease in lysine content was observed following the fourth Edman degradative step. Further verification of this sequence was obtained by treatment of LT3C4 with leucine aminopeptidase followed by dansyl-Edman sequence analysis. The sequence of LT3 determined by these means is presented in Table IV.

The complete sequence of LT7 was determined in a similar manner, and the results are summarized in Table V. Peptides LT7C1-LT7C5 (Table VI) were isolated from a chymotryptic digest of LT7 by gel filtration on Sephadex G-25 and Sephadex G-50 in 0.015 N NH_4OH and ion-exchange chromatography of the peptide mixture obtained on AG50. Peptides LT7C1-LT7C5 account for 31 of the 35 residues of LT7. A peptide representing the remaining amino acids (Glx_2, CMCys, and Tyr) was not isolated from the digest, but a peptide (LC-11, Table VII) with the sequence Tyr-CMCys-Glx-Glx-Tyr was isolated from a chymotryptic digest of L_1 and L_2 mixtures.

Order of the Tryptic Peptides of L_1 and L_2. The tryptic peptides LT1′, LT1B, LT5A, LT5B, and LT8A can be positioned in the cyanogen bromide fragments L_1 and L_2 on the basis of the information summarized in Figure 1. Peptide LT1′ is the amino-terminal tryptic peptide of L_1, and LT1B represents residues 4–18 of L_1. Peptide LT5A is the carboxyl-terminal peptide of L_1, and LT5B is the amino-terminal peptide of L_2. The isolation of peptide LO-2 (Waxdal *et al.*, 1968b) from a tryptic digest of the whole light chain and the isolation of peptide LT8A (free homoserine) from a tryptic digest of a mixture of L_1 and L_2 places LT8A at the carboxyl-terminal position of L_2 adjacent to a lysine or arginine residue.

In order to place the remaining peptides of L_1 and L_2, a mixture of these CNBr fragments was digested with chymotrypsin, and the resulting peptides were isolated. The compositions of these peptides are presented in Table VII. The peptides listed in Table VII were initially fractionated by gel filtration on Sephadex G-25 in 5% formic acid (Figure 5). Gel filtration of fraction A on Sephadex G-50 in 2% acetic acid followed by filtration on Sephadex G-25 in the same solvent and ion-exchange chromatography on AG50X4 yielded peptides LC1, LC2, LC6, LC7, LC8, and LC9. Fraction B yielded peptide LC3 when treated in a similar fashion. Fraction C contained a mixture of peptides which was not fractionated further. Fraction D contained a mixture of peptides LC10 and LC11 which was resolved by gel filtration on Sephadex G-25 in *n*-propyl alcohol–acetic acid–water (1:1:3, v/v). Fractions E and F yielded peptides LC4 and LC5, respectively, without further purification.

TABLE III: Amino Acid Composition of Chymotryptic Peptides of LT3.[a]

	LT3C1	LT3C2	LT3C3	LT3C4
Lys				2.1 (2)
Asp	1.0 (1)			
Thr	1.0 (1)			
Ser	1.7 (2)		0.3	
Glu	1.2 (1)			1.9 (2)
Pro				1.0 (1)
Gly				1.0 (1)
Ala	0.9 (1)	1.0 (1)	1.0 (1)	
Ile	0.9 (1)			
Leu		1.0 (1)	1.0 (1)	
Tyr			0.8 (1)	0.4
Trp	(1)	(1)	(1)	
Total residues	8	3	4	6
Yield[b] (%)	56	39	12	57

[a] Values reported are amino acid residues. Amino acids present at a level less than 0.2 residue are omitted. Values in parentheses are nearest integral numbers of residues. [b] Yields are based on micromoles of peptides isolated compared with micromoles of LT3 digested with chymotrypsin.

TABLE IV: Amino Acid Sequence of Tryptic Peptide LT3.

LT3C1[a]	Ala-Ser-Glx-Ser-Ile-Asx-Thr-Trp
LT3C2[a]	Leu-Ala-Trp
LT3C3[a]	(Leu,Ala,Trp)Tyr
LT3C4	(Glx,Glx,Lys,Pro,Gly)Lys
LC6	Tyr-Glx-Glx-Lys(Pro)Gly(Lys,Ala,Pro,Lys,Leu,Leu)Hsr
LT3[b]	Ala-Ser-Glx-Ser-Ile-Asx-Thr-Trp-Leu-A'a-Trp-Try-Glx-Glx-Lys-Pro-Gly-Lys

[a] Carboxyl-terminal tryptophan and tyrosine determined by carboxypeptidase A. [b] The complete sequence of LT3 is deduced from these data and the partial sequence of LT3 as presented in Table II.

TABLE V: Amino Acid Sequence of Tryptic Peptide LT7.

LT7C1	Phe-Ile-Gly-Ser-Gly-Ser-Gly-Thr-Glx-Phe
LT7C2	Ile-Gly-Ser(Gly,Ser,Gly,Thr,Glx,Phe)
LT7C3	Thr-Leu-Thr-Ile-Ser-Ser-Leu-Glx-Pro-Asx-Asx-Phe-Ala-Thr-Tyr
LT7C4[a]	(Thr,Leu,Thr,Ile,Ser,Ser,Leu,Glx,Pro,Asx,Asx,Phe,Ala,Thr)Tyr-Tyr
LC11	Tyr-CMCys-Glx-Glx-Tyr
LT7C5	Asx-Ser-Asx-Ser-Lys
LT7[b]	Phe-Ile-Gly-Ser-Gly-Ser-Gly-Thr-Glx-Phe-Thr-Leu-Thr-Ile-Ser-Ser-Leu-Glx-Pro-Asx-Asx-Phe-Ala-Thr-Tyr-Tyr-CMCys-Glx-Glx-Tyr-Asx-Ser-Asx-Ser-Lys

[a] The carboxyl-terminal Tyr-Tyr sequence was established by carboxypeptidase digestion. [b] The complete sequence of LT7 is based on these data and on the portions of LT7 presented in Table II.

TABLE VI: Amino Acid Composition of Chymotryptic Peptides of LT7.[a]

	LT7C1	LT7C2	LT7C3	LT7C4	LT7C5
Lys					1.0 (1)
Asp			2.0 (2)	2.0 (2)	2.0 (2)
Thr	1.0 (1)	1.0 (1)	2.8 (3)	2.8 (3)	
Ser	1.8 (2)	2.1 (2)	1.8 (2)	1.9 (2)	1.9 (2)
Glu	1.1 (1)	1.1 (1)	1.1 (1)	1.1 (1)	0.4
Pro			1.2 (1)	1.1 (1)	
Gly	3.0 (3)	2.9 (3)			0.4
A'a			0.8 (1)	0.9 (1)	
Ile	1.0 (1)	0.8 (1)	1.0 (1)	1.0 (1)	
Leu			1.9 (2)	1.9 (2)	
Tyr			0.8 (1)	1.6 (2)	
Phe	2.0 (2)	0.8 (1)	1.0 (1)	1.0 (1)	
Total residues	10	9	15	16	5
Yield[b] (%)	12	5	48	23	26

[a] Values presented are amino acid residues. Amino acids present at a level less than 0.2 residue are not given. Values in parentheses represent nearest integral numbers of residues. [b] Yields are based on micromoles of peptides isolated compared with micromoles of LT7 digested with chymotrypsin.

The amino acid compositions (Table VII) and partial sequences of the chymotryptic peptides (Table VIII) permits the ordering of the tryptic peptides of L_1 and L_2 as summarized in Figure 6. The carboxyl-terminal sequence of LC7 (Arg-Phe) was determined by digestion with carboxypeptidases A and B.

The Amino Acid Sequence of L_3. Direct sequence analysis of CNBr fragment L_3 by the dansyl-Edman pro-

TABLE VII: Amino Acid Composition of Chymotryptic Peptides from L_1 and L_2 Mixture.[a]

	LC1	LC2	LC3	LC4	LC5	LC6	LC7	LC8	LC9	LC10	LC11
Lys						2.9 (3)	1.2 (1)				
Arg		1.1 (1)	1.1 (1)				1.0 (1)				
Hsr[b]	0.7 (1)					1.0 (1)					0.7 (1)
CMCys		0.7 (1)							2.1 (2)		
Asp	1.0 (1)	1.2 (1)	1.0 (1)					1.1 (1)	2.7 (3)		
Thr	1.9 (2)	1.8 (2)	1.0 (1)				3.3 (4)	2.2 (2)	2.1 (2)		
Ser	1.9 (2)	2.1 (2)	2.0 (2)			2.0 (2)	1.0 (1)	1.1 (1)	1.2 (1)		2.1 (2)
Glu	2.0 (2)		1.0 (1)			1.9 (2)	1.0 (1)		1.0 (1)		
Pro	1.0 (1)					1.1 (1)	1.0 (1)	2.9 (3)			
Gly		1.0 (1)				0.9 (1)	0.9 (1)		0.8 (1)		
Ala		0.9 (1)	0.9 (1)	1.0 (1)	1.0 (1)						
Cys		0.2					0.9 (1)				
Val		1.9 (2)						0.9 (1)	1.0 (1)		
Ile	0.9 (1)	0.9 (1)	0.9 (1)						1.9 (2)		
Leu	1.0 (1)			1.0 (1)	1.0 (1)	1.9 (2)	1.0 (1)		0.8 (1)	1.0 (1)	1.9 (2)
Tyr					1.2 (1)	0.7 (1)	0.6 (1)				
Phe							0.9 (1)	1.0 (1)	1.0 (1)		
Trp			(1)	(1)	(1)						
Total residues	11	12	9	3	4	13	14	9	15	1	5
Yield[c] (%)	63	21	14	37	16	10	19	47	37	21	49

[a] Values are amino acid residues. Amino acids present at a level less than 0.2 residue are not included. Values in parentheses are nearest integral numbers of residues. [b] Includes homoserine and homoserine lactone. [c] Yields are based on micromoles of peptides isolated compared with micromoles of L_1 and L_2 digested with chymotrypsin.

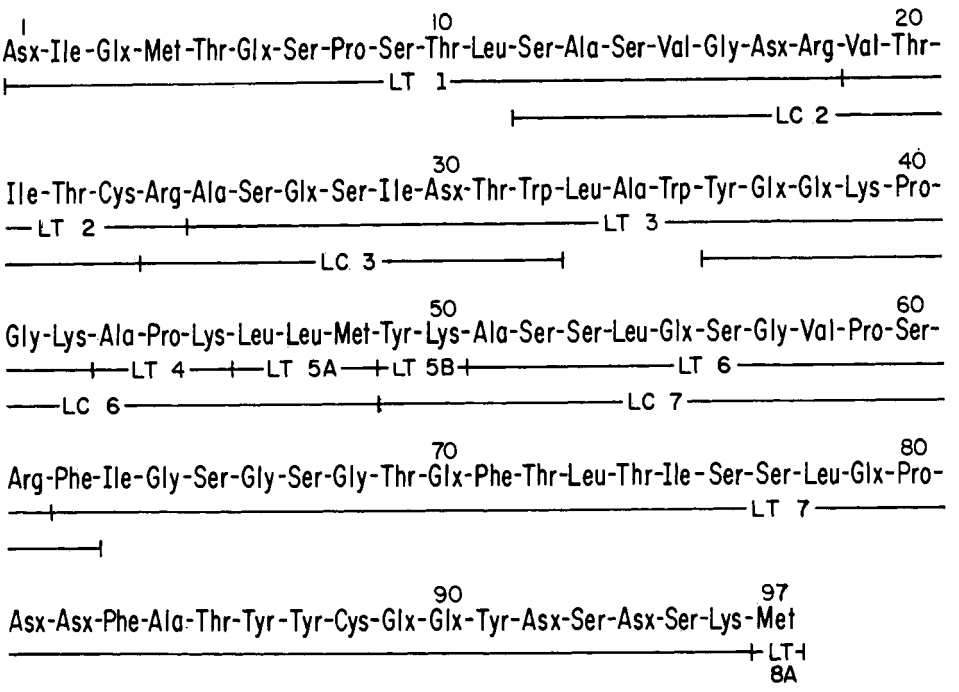

FIGURE 6: Amino acid sequence of the portion of the variable region of Eu light chain represented by fragments L_1 and L_2. Tryptic peptides LT1-LT8A were ordered by means of information presented in Figure 1 in addition to the partial sequences of chymotryptic peptides LC2, LC3, LC6, and LC7 (Table VIII).

cedure indicated that the first eight residues are: Phe-Gly-Glx-Gly-Thr-Lys-Val-Glx-. Additional information about the amino acid sequence of the region corresponding to this fragment was provided by the isolation of tryptic peptides from the whole light chain. The initial fractionation of soluble tryptic peptides obtained from the intact light chain by ion-exchange chromatography on AG50X4 is presented in Figure 7, and the amino acid compositions of the peptides isolated from this digest are presented in Table IX. Peptides LT6, LT8, LT13, and LT20 were obtained from peaks J, T, Z, and G (Figure 7), respectively, and required no further purification. Peptide LT17 was obtained by extensive washing of the column with 2 M pyridine adjusted with acetic acid to pH 5.5. The majority of peptides listed in Table IX was isolated by ion-exchange chromatography of fractions B (LT15), D (LT1 and LT 14), H (LT15), P (LT16 and LT18), Q (LT9 and LT12), and X (LT5 and LT19) on AG1X4. Ion-exchange chromatography of fraction L on AG50X4 yielded LT11. The yields of peptides LT1, LT11, and LT14 were low, and few of the peptides from the L_1 and L_2 region were isolated. However, about 50% of the digested material was insoluble and was not fractionated. Many of the peptide mixtures obtained from fractionation of the soluble peptides were too complex to be purified readily.

Peptide LT6 (Table IX) was also isolated from L_1 and L_2. Peptide LT1, mentioned earlier, is equivalent to peptide LT1′ obtained from L_1 and L_2 and represents the amino-terminal 18 residues of the light chain. Peptide LT5 is overlap peptide LO-1 (Figure 1), and LT8 is overlap peptide LO-2 (Figure 1) (Waxdal *et al.*, 1968a).

The amino acid sequences of LT9, LT12, LT13, LT16, LT17, LT19, and LT20 were determined, and the results

TABLE VIII: Partial Amino Acid Sequences of Chymotryptic Peptides of L_1 and L_2.

LC1	Asx-Ile-Glx-Hsr-Thr-Glx(Ser,Pro,Ser,Thr,Leu)
LC2	Ser-Ala-Ser-Val(Gly,Asx,Arg,Val,Thr,Ile,-Thr,CMCys)
LC3	Arg-Ala-Ser-Glx-Ser-Ile-Asx-Thr-Trp
LC6	Tyr-Glx-Glx-Lys(Pro)Gly(Lys,Ala,Pro,Lys,-Leu,Leu)Hsr
LC7[a]	Tyr-Lys-Ala-Ser-Ser-Leu-Glx-Ser(Gly,Val,-Pro,Ser)Arg-Phe

[a] The COOH-terminal Arg-Phe sequence was determined by treatment with carboxypeptidases A and B.

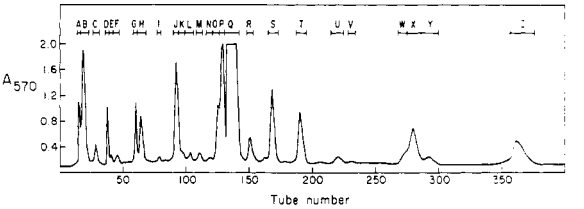

FIGURE 7: Initial fractionation of the soluble tryptic peptides from the whole light chain by ion-exchange chromatography on a column (0.9 × 180 cm) of AG50X4 at 60°. A linear gradient of 350 ml each of 0.1 M pyridine (pH 3.1) and 2.0 M pyridine (pH 5.5) was employed. Following completion of the gradient, the column was washed overnight with the final buffer. The column effluent was monitored continuously for ninhydrin-positive material (Waxdal *et al.* 1968b) and the absorbance at 570 mμ is represented by the solid line. Each tube contained 3.0 ml of effluent.

TABLE IX: Amino Acid Composition of Tryptic Peptides from Whole Eu Light Chain.[a]

	LT-1	LT-5	LT-6	LT-8	LT-9	LT-10	LT-11	LT-12	LT-13	LT-14	LT-15	LT-16	LT-17	LT-18	LT-19	LT-20
Lys		0.9 (1)		1.0 (1)	0.9 (1)	1.0 (1)		0.9 (1)	0.9 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)	1.0 (1)		
His													1.0 (1)	1.0 (1)		
Arg	1.0 (1)		0.9 (1)				0.8 (1)								1.0 (1)	
CMCys							0.5 (1)							0.9 (1)		0.8 (1)
Asp	1.9 (2)					1.0 (1)	2.0 (2)			4.0 (4)	1.2 (1)	0.9 (1)			1.0 (1)	
Thr	1.9 (2)			1.0 (1)		1.0 (1)	1.1 (1)			1.1 (1)	3.1 (3)			1.9 (2)		
Ser	3.3 (4)		3.5 (4)			1.8 (2)	1.8 (2)			3.7 (4)	4.8 (5)			1.9 (2)	0.8 (1)	
Glu	1.9 (2)		1.2 (1)	1.1 (1)	1.1 (1)	2.0 (2)		1.1 (1)	1.1 (1)	5.0 (5)		1.1 (1)		2.2 (2)		1.1 (1)
Pro	0.9 (1)		1.1 (1)			2.8 (3)	1.1 (1)							1.0 (1)		
Gly	1.0 (1)		0.9 (1)	1.9 (2)		1.0 (1)	1.3 (1)			1.1 (1)				1.1 (1)		0.9 (1)
Ala	1.0 (1)		1.0 (1)			1.9 (2)	1.0 (1)	1.0 (1)		1.0 (1)		0.9 (1)		1.0 (1)		
Cys							0.2									0.3
Val	0.9 (1)		1.1 (1)		1.9 (2)	2.0 (2)	1.6 (2)		1.1 (1)	2.0 (2)				3.0 (3)		
Met	1.0 (1)	1.1 (1)		0.9 (1)												
Ile	1.1 (1)					1.0 (1)										
Leu	1.1 (1)	2.1 (2)	1.1 (1)			1.0 (1)	2.5 (2)			1.0 (1)	3.0 (3)			1.0 (1)		
Tyr		0.9 (1)					0.9 (1)				0.7 (1)	0.8 (1)		0.9 (1)		
Phe				1.0 (1)		2.0 (2)	1.0 (1)								1.1 (1)	
Trp									(1)							
Total residues	18	5	11	7	4	19	16	3	4	20	14	5	2	17	4	3
Yield[b] (%)	3	20	34	16	33	32	1	33	97	2	9	26	62	9	18	44

[a] Values reported are amino acid residues. Amino acids present at a level less than 0.2 residue are omitted. Values in parentheses are nearest integral numbers of residues. [b] Yields are based on micromoles of peptides isolated compared with total micromoles of light chain digested with trypsin.

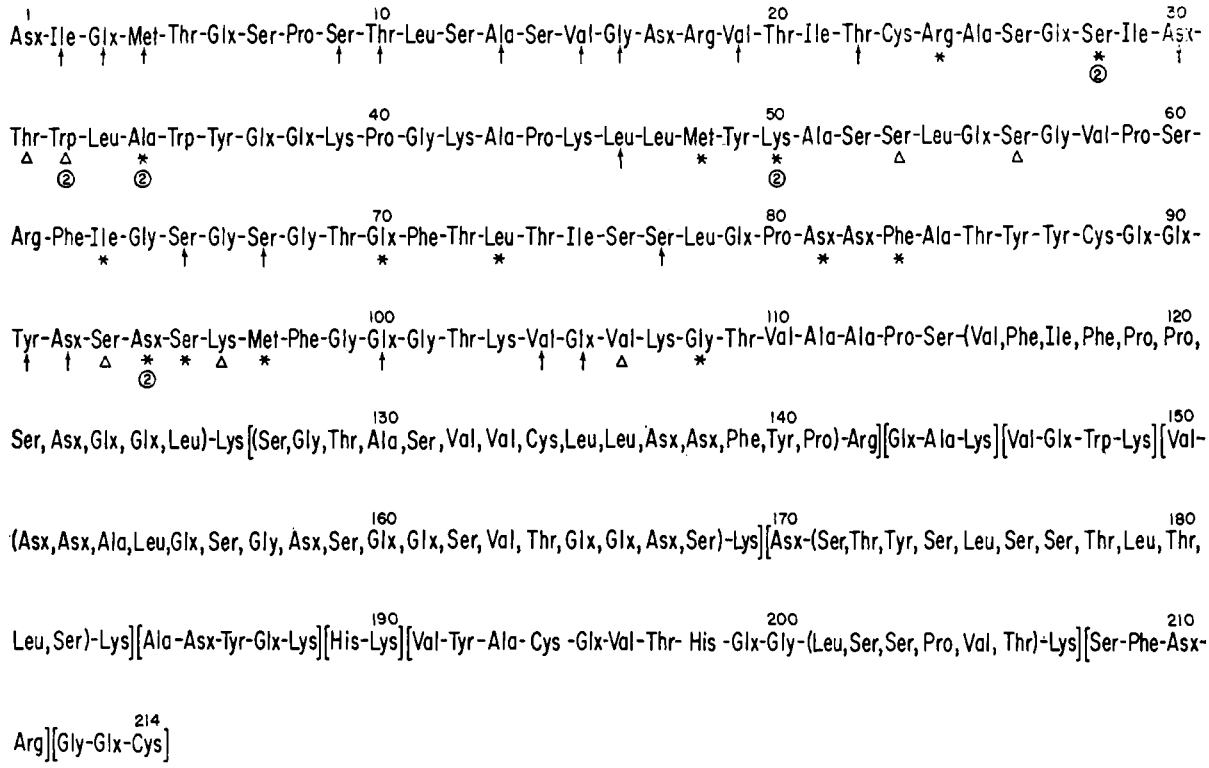

FIGURE 8: Partial amino acid sequence of the Eu light chain. The symbol ↑ denotes a variable position of κ chains at which the residue found in Eu has been previously observed. The notation Δ indicates a position known to vary in κ chains, but at which the residue observed in the sequence of Eu has not been previously observed. An asterisk (*) beneath a residue in the sequence marks new variable positions in κ chains. The notation ② under a symbol indicates that a two-base change is required in the codons specifying the amino acids previously seen at this position in order to obtain the residue observed in Eu. Brackets enclose tryptic peptides whose position in the chain has not been definitely established. Parentheses enclose residues whose sequence has not yet been determined. Bracketed peptides are all in the constant region, and their position is based on homology with the known sequence of κ chains.

are presented in Table X. Because of their large size, LT10, LT11, LT14, LT15, and LT18 were not sequenced entirely; the partial sequences of LT10 and LT18 and the amino-terminal residues of LT14 and LT15 are presented in Table X. For purposes of comparison, the sequence of the amino-terminal eight residues of L_3 is also included in this table.

Peptide LT8 (LO-2) contains the amino-terminal tryptic peptide of L_3. Because LT20 was the only peptide isolated from the tryptic digest of the light chain that did not contain lysine or arginine and was obtained in good yield, this peptide was placed at the carboxyl terminus of L_3.

A peptide designated L3C1 of composition $Phe_{1.9}$,-$Val_{3.9}$,$Ala_{1.9}$,$Gly_{2.7}$,$Pro_{1.3}$,$Glu_{2.2}$,$Ser_{1.0}$,$Thr_{1.9}$,$Lys_{2.2}$ was isolated from a chymotryptic digest of L_3 in 38% yield by ion-exchange chromatography on AG-50X4. The partial sequence of this peptide was determined as: Phe-Gly-Glx-Gly-Thr-Lys-Val-Glx-Val-Lys-Gly(Thr,Val,Ala,Ala,Pro,Ser,Val,Phe). This peptide overlaps LT8, LT9, and LT10. The remaining peptides are positioned in L_3 on the basis of homology with the known sequences of other κ chains (Titani *et al.*, 1967; Hilschmann and Craig, 1965; Hilschmann, 1967; Gray *et al.*, 1967). The sequence of the Eu light chain based on all of the data described above is presented in Figure 8.

Discussion

The presence of three methionine residues in the variable portion of the Eu light chain permits the separation of most of this region from the constant region. The prospect of comparing a variable region of a light chain with that of the heavy chain from the same myeloma globulin and with urinary Bence–Jones proteins prompted us to determine the amino acid sequence of CNBr fragments L_1 and L_2 and a sufficient number of the amino-terminal residues of L_3 to establish the sequence of the entire variable region of the light chain.

Human κ chains have been classified into three subgroups mainly on the basis of the sequence of their amino-terminal 23 residues (Milstein, 1967; Niall and Edman, 1967). Fewer differences in sequence are seen within each subgroup than between subgroups. Eu light chain fits into the subgroup containing Bence–Jones proteins Ag (Titani *et al.*, 1967) and Roy (Hilschmann, 1967), and it differs in sequence from these two proteins in 26 and 27, respectively, of the first 108 residues. The sequences of Ag and Roy differ in only 14 positions.

Comparison of the sequence of the first 108 residues of Eu light chain with the sequences of κ chains of the same subgroup indicates a high degree of variability in Eu. Fourteen positions (Figure 8) in Eu contain amino acid substitutions which have never before been seen at

TABLE X: Amino Acid Sequence of Tryptic Peptides from Whole Eu Light Chain.

L3	Phe-Gly-Glx-Gly-Thr-Lys-Val-Glx-
LT9	Val-Glx-Val-Lys
LT10	Gly-Thr-Val-Ala-Ala-Pro-Ser(Val,Phe,Ile,-Phe,Pro,Pro,Ser,Asx,Glx,Glx,Leu)Lys
LT12	Glx-Ala-Lys
LT13	Val-Glx-Trp-Lys
LT14	Val(Asx,Asx,Ala,Leu,Glx,Ser,Gly,Asx,Ser,-Glx,Glx,Ser,Val,Thr,Glx,Glx,Asx,Ser)Lys
LT15	Asx(Ser,Thr,Tyr,Ser,Leu,Ser,Ser,Thr,Leu,-Thr,Leu,Ser)Lys
LT16	Ala-Asx-Tyr-Glx-Lys
LT17	His-Lys
LT18	Val-Tyr-Ala-CMCys-Glx-Val-Thr-His-Glx-Gly(Leu,Ser,Ser,Pro,Val,Thr)Lys
LT19	Ser-Phe-Asx-Arg
LT20	Gly-Glx-CMCys

these positions. Of these, four require two-base changes in the codons which specify amino acids usually seen in these positions in other proteins. Seven substitutions in Eu occur at positions known to be variable, but the particular amino acids observed in Eu light chain have not previously been found at these positions. One of these seven substitutions (Trp 32) requires a two-base change in a codon. At 20 positions known to be variable, Eu light chain contains amino acids which have previously been observed. The remaining 67 of the first 108 residues of Eu light chain are identical with residues which have not been observed to vary in κ proteins of this subgroup. In all these comparisons, we have assumed a complete correspondence between the amides of Eu κ chains and those of Bence–Jones proteins.

The sequence of the carboxyl-terminal portion of peptide LT3, Tyr-Glx-Glx-Lys-Pro-Gly-Lys-, differs from that reported for the Bence–Jones proteins Roy (Hilschmann and Craig, 1965; Hilschmann, 1967) and Ag (Titani *et al.*, 1967) although the amino acid compositions are identical. The variations arising from this difference are not included in the comparison presented above.

Amino acid compositions and partial sequence analyses of L_3 indicate that with the notable exception of residue 108, the constant region of Eu light chain is identical with the constant region of Bence–Jones proteins of the same antigenic class and Inv specificity. Eu has been typed as Inv 3 and contains a valine at position 191. Sequence analysis of the amino-terminal portion of L_3 and of peptides L3C1 and LT10 establish conclusively that residue 108 of Eu light chain is glycine. In all other κ chains, this position is occupied by arginine and has been considered to be the first residue of the constant region. We suggest that either position 108 is part of the variable region or it is a site of genetic variation similar to that observed for the Inv locus (Baglioni *et al.*, 1966).

We have previously noted the failure of CNBr consistently to cleave the methionine at position 4 (Waxdal *et al.*, 1968b). This is possibly the result of alkylation of the methionine by iodoacetamide during the preparation of light chains. Sequence analysis by the dansyl-Edman technique of light chain prepared by alkylation with iodoacetamide yielded two fluorescent spots at position 4. One of these was dansylmethionine; the other was not identified, but was not dansylhomoserine, dansylhomoserine lactone, dansylmethionine sulfone, or dansylmethionine sulfoxide. Sequence analysis by the dansyl-Edman technique of light chain prepared by alkylation with *N*-ethylmaleimide yielded only dansylmethionine in position 4.

The homology between the variable regions of Eu light chain and Bence–Jones proteins of the same antigenic class (κ) and subgroup is obvious. As discussed above, the sequence of the constant portion of Eu light chain (excepting position 108) is probably identical with that of κ Bence–Jones proteins. These observations are consistent with the previous conclusion (Edelman and Gally, 1962; Schwartz and Edelman, 1963) that urinary Bence–Jones proteins are light chains.

The data presented in this report provide a basis for comparing the primary structure of the light chain of the myeloma protein, Eu, with that of the Eu heavy chain. This will be the subject of a future paper in this series.

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The Catalytic Versatility of Erythrocyte Carbonic Anhydrase. V. Kinetic Studies of Enzyme-Catalyzed Hydrations of Aliphatic Aldehydes*

Y. Pocker† and D. G. Dickerson

ABSTRACT: The present investigation demonstrates that bovine carbonic anhydrase is a powerful catalyst for the reversible hydrations of propionaldehyde and isobutyraldehyde. The enzyme does not catalyze the hydration of pivalaldehyde, and, furthermore, it does not even appear to bind detectable amounts of this aldehyde. Hydrase activity rises with pH between 4.3 and 8; although the pH profiles for both propionaldehyde and isobutyraldehyde are sigmoidal, the former is characterized by an inflection around pH 6.6 whereas the inflection obtained with isobutyraldehyde is around 5.6. The profile for propionaldehyde reflects the variation in the turnover number with pH. Acetazolamide functions as a noncompetitive inhibitor of enzyme activity with propionaldehyde as substrate but serves as a competitive inhibitor of markedly diminished inhibitory capacity in the enzymic hydration of isobutyraldehyde. Thiocyanate and azide anions, while acting as noncompetitive inhibitors with propionaldehyde, are without detectable effect on the enzymic hydration of isobutyraldehyde.

Various aliphatic alcohols inhibit both reactions in a competitive fashion. Several similarities were noted between the binding of aldehydes and alcohols, most significant perhaps was the observation that *t*-butyl alcohol, in analogy with pivalaldehyde, does not appear to bind to bovine carbonic anhydrase. Observations relevant to the binding step are interpreted as being indicative of the importance of hydrophobic interactions in substrate binding. The data are analyzed in terms of a hydrating site of variable conformation depending on the nature of the substrate and containing an imidazole residue, the bound substrate, and protein-bound zinc–aquo complex.

It has been demonstrated in these laboratories that erythrocyte carbonic anhydrase (carbonate hydrolyase EC 4.2.1.1) (CA)[1] is not, as has been previously thought, an absolutely specific catalyst for the reversible hydration of carbon dioxide, a catalysis which does indeed embody its physiological function, but it also powerfully catalyzes the reversible hydrations of acetaldehyde and various pyridine aldehydes (Pocker and Meany, 1965a,b, 1967a; Pocker *et al.*, 1965). In addition, it has been demonstrated that carbonic anhydrase is a very potent esterase with respect to a variety of esters of *o*- and *p*-nitrophenol (Pocker and Stone, 1965, 1967; Pocker and Storm, 1968; *cf.* also Tashian *et al.*, 1964;

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† Author to whom correspondence should be addressed.

[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: CA, carbonic anhydrase; BCA, bovine carbonic anhydrase.