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Mouse Immunoglobulin Chains. Partial Amino Acid Sequence of a κ Chain[†]

David McKean,[‡] Michael Potter, and Leroy Hood^{*·§}

ABSTRACT: The nearly complete amino acid sequence from the variable region (residues 1–107) of BALB/c mouse κ chain MOPC 321 is presented. Compositional data are presented on tryptic, thermolytic, and chymotryptic peptides derived from the constant region (residues 108–214). Methionine residues at positions 33 and 175 permitted this protein to be cleaved with cyanogen bromide into three fragments which were separated in part by gel filtration. The cyanogen bromide

fragments were subsequently subjected to enzymatic cleavage and the resulting peptides were isolated on paper by chromatography and electrophoresis. The automatic sequenator was used to confirm the sequence of the amino-terminal 37 residues. The combination of fortuitous methionine placement, isolation of peptides from paper, and the sequenator analysis permitted this protein to be analyzed very rapidly.

The previous paper in this series demonstrated that certain mouse immunoglobulin κ chains¹ are identical or nearly identical in amino-terminal sequence for 23 residues (Hood *et al.*, 1973). A comparative peptide map analysis of one group of these mouse κ chains, M321, M63, and T124,² showed that each of these light chains is similar but not identical with the others. We report here the nature of amino acid sequence differences which exist within this group of similar mouse immunoglobulin κ chains. This paper is a report on the methodology employed to sequence these proteins and describes in detail the analysis of one protein, M321. The following paper (McKean *et al.*, 1973) will present the two additional

partial sequences from this group of proteins and will discuss the implications of these sequence comparisons with regard to genetic and evolutionary mechanisms of antibody diversity.

Materials and Methods

Protein Isolation. M321 was isolated from the urine of female BALB/c mice bearing the corresponding plasma cell tumor. The urine was clarified by centrifugation and exhaustively dialyzed against 0.1 M ammonium bicarbonate. The mouse Bence-Jones protein was separated from mouse urinary protein (MUP)³ by ion-exchange chromatography on a DEAE-A25 column equilibrated with 0.05 M Tris-acetate buffer at pH 5.5 (Potter, 1967). The light chain is eluted with this buffer whereas the MUP is bound.

Protein Purity. The light-chain purity was determined by agar gel electrophoresis at pH 8.2 (Potter and Kuff, 1964) (see Figure 1) and by Ouchterlony analysis with rabbit anti-MUP antiserum.

Protein Preparation. Purified light chains were dissolved in 7 M guanidine-HCl–0.2 M Tris-HCl (pH 8.6) at a concentration of 20 mg/ml. Intrachain disulfide bonds were reduced with 50 mM dithiothreitol at 37° for 90 min. An equal volume of 0.11 M iodoacetamide dissolved in 7 M guanidine-HCl–0.2

[†] From the Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218 (D. M.), from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014 (M. P.), and from the Division of Biology, California Institute of Technology, Pasadena, California (L. H.). Received August 18, 1972.

[‡] Present address: Department of Medical Genetics, University of Wisconsin, Madison, Wis. 53706. This work was in part carried out at the National Cancer Institute while the senior author was a guest worker, in fulfillment for a doctoral dissertation.

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¹ The abbreviations used for immunoglobulin chains are those proposed by the World Health Organization: see *Bull. W. H. O.* 41, 975 (1969).

² Mouse immunoglobulin chains MOPC 321, MOPC 63, TEPC 124, and MOPC 70 are abbreviated M321, M63, T124, and M70, respectively.

³ Abbreviations used are: MUP, mouse urinary protein; PTH, phenylthiohydantoin; dansyl, 1 dimethylaminonaphthalene-5-sulfonyl.

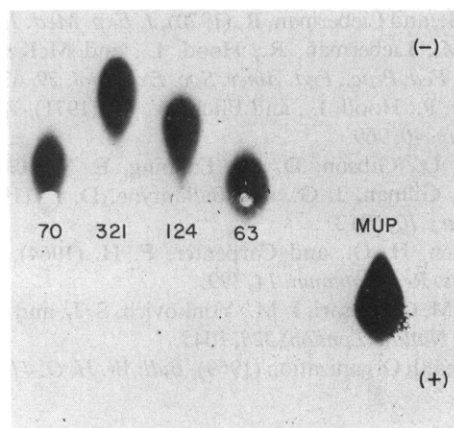


FIGURE 1: Agar gel electrophoresis of four mouse Bence-Jones proteins and mouse urinary protein (MUP). These proteins were run in 1% agar at pH 8.6 in 0.05 M Tris-acetate buffer at 40 V for 2 hr.

M Tris-HCl (pH 7.3) was added and the reaction was carried out at 25° for 15 min. The solution was then dialyzed against repeated changes of water at 4° or run through a G-10 Sephadex column equilibrated with 1 N acetic acid.

Aminoethylation was carried out essentially as described by Raferty and Cole (1963) except that the proteins were initially dissolved in 0.2 M Tris-HCl and 7 M guanidine-HCl at pH 8.6.

Amino Acid Analysis. Peptides were hydrolyzed in evacuated tubes (<0.02 torr) in 5.7 N (constant-boiling) HCl at 110° for 24 hr. Protein samples (native and alkylated) were hydrolyzed for 24 and 48 hr and the appropriate corrections were made. Quantitative analyses were performed with the zinc-ligand-exchange system using the Hitachi Perkin-Elmer Model KLA and with the Beckman Model 121 amino acid analyzers. Qualitative analyses were carried out by high-voltage electrophoresis on paper (Dreyer and Bynum, 1967).

Tryptophan content was determined by acid hydrolysis in 4% thioglycolic acid (Matsubara and Sasaki, 1969) which gave recoveries up to 70%. Erlich's stain was also used to identify tryptophan-containing peptides. Aminoethylcysteine was estimated semiquantitatively by peak height because of its incomplete separation from ammonia. Homoserine could be distinguished from serine on the Beckman system but not on the zinc-ligand system.

Enzymes. Trypsin treated with L-(tosylamido-2-phenyl)-ethyl chloromethyl ketone and chymotrypsin (three-times recrystallized) were purchased from Worthington Biochemical Corp. and stored as 1% solutions in 0.001 N HCl at -20°. Carboxypeptidases A and B, treated with diisopropyl fluorophosphate, were also purchased from Worthington. Carboxypeptidase A (50 mg) was washed three times in 2 ml of water and dissolved in 2.0 M ammonium bicarbonate at 20 mg/ml. A slight precipitate was spun down and 20-μl aliquots were placed in small tubes (0.5 × 5 cm), frozen, sealed under N₂, and stored at -20° until just prior to use. Carboxypeptidase B was stored in the same manner as carboxypeptidase A. Thermolysin (three-times recrystallized) was purchased from California Biochemical Corp. and freshly prepared as 1% solution in distilled water before each digestion.

Edman Reagents. Pyridine, ethyl acetate, and butyl acetate (reagent grade) were redistilled over ascorbic acid prior to use in the dansyl-Edman procedure. Trifluoroacetic acid (Sequal grade) was purchased from Pierce Chemical Corp. Phenyl

TABLE I: Amino Acid Composition of M321.

	M321
Asp	26 ^a (25) ^b
Glu	20 (21)
Thr	20 (20)
Ser	28 (30)
Pro	16 (12)
Ala	13 (13)
Gly	13 (12)
Val	11 (11)
Met	2 (2)
Ile	10 (10)
Leu	15 (13)
Tyr	10 (8)
Phe	8 (9)
Lys	12 (12)
His	3 (2)
Arg	9 (9)

^a Values reported are amino acid residues from 24-hr hydrolysates of native light chain rounded off to the nearest integral number except for Ser and Thr values which are based on a linear extrapolation of 24- and 48-hr hydrolysis values to zero time. Cys and Trp were not quantitated. ^b Expected residue values from the partial sequence of M321 are presented in parentheses.

isothiocyanate was obtained from Eastman Chemical Co. and redistilled *in vacuo*. All reagents were stored at -20° under N₂.

Sequencer reagents and solvents were purchased from Beckman Instruments Co.

PTH-amino acids were purchased from Mann Research Laboratories.

Dansyl Reagents and Materials. Dansyl-Cl and dansyl-amino acids were obtained from Pierce Chemical Co. Polyamide thin-layer chromatography sheets were purchased from Cheng Chin Trading Co., Ltd. (Gallard-Schlesinger, Chemical Manufacturing Co.). The formic acid, benzene, glacial acetic acid, ethyl acetate, and methanol used in dansyl-amino acid thin-layer chromatography were reagent grade.

Other Reagents. Dithiothreitol was purchased from California Biochemical Corp. and used without further purification. Iodoacetamide (Pierce Chemical Corp.) was recrystallized three times from ethanol. [¹⁴C]Iodoacetamide was purchased from New England Nuclear Corp.

Ethylenimine was purchased from Matheson Coleman and Bell Co. Tris (Ultra Pure) was obtained from Schwarz Bio-Research. Guanidine-HCl (Ultra Pure) was obtained from Mann Research. Reagent grade cyanogen bromide was obtained from Eastman Chemical Co.

Ehrlich Stain. Ehrlich stain was prepared and used as described by Smith (1953).

Enzyme Digestions. Light chains which had been completely reduced and alkylated or aminoethylated were suspended in 0.2 M ammonium bicarbonate (10 mg/ml) and digested with 2% (w/w) trypsin at 37° for 4-16 hr.

Completely reduced and alkylated light chains were digested in 0.2 M NH₄HCO₃ with thermolysin and chymotrypsin (1% w/w) at 37° for 2 hr.

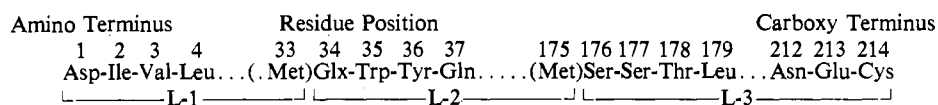


FIGURE 2: Alignment of cyanogen bromide fragments from M321 (see text). The numbering is that of Gray *et al.* (1967).

TABLE II: Amino Acid Composition of Tryptic Peptides from CN-C.^a

	C-T2	C-T3	C-T4	C-T19	C-T20	C-T21	C-T22	C-T23	C-T24
Tyr			0.8 (1)		0.7 (1)	0.9 (1)			
Phe			0.9 (1)					1.0 (1)	
Lys		1.1 (1)		0.9 (1)		1.0 (1)	1.0 (1)		
His						1.9 (2)			
Arg	0.9 (1)				1.2 (1)			1.0 (1)	
Asp			2.2 (2)		1.0 (1)	1.1 (1)		1.2 (1)	0.9 (1)
Glu					2.2 (2)	1.0 (1)			1.1 (1)
Thr	1.1 (1)		0.9 (1)	3.2 (3)		1.9 (2)	2.2 (2)		
Ser	1.0 (1)	0.9 (1)	2.4 ^b (3)	1.9 (2)		0.9 (1)	1.8 (2)	0.9 (1)	
Pro							1.0 (1)		
Ala	1.1 (1)	0.9 (1)				1.0 (1)			
Gly			1.1 (1)						
Val			1.0 (1)				1.1 (1)		
Met									
CMCys	0.8 (1)					0.8 (1)			0.8 (1)
Ile	0.9 (1)						1.0 (1)		
Leu				2.0 (2)					
Trp									
Total residues	6	3	10	8	5	11	8	4	3
Yield (%) ^c	15	15	14	9	18	16	14	36	15

^a Values reported are amino acid residues. Amino acids present at a level of less than 0.2 residue are omitted. Values in parentheses represent the nearest integral number of residues. ^b Includes homoserine as well as serine as the zinc ligand amino acid analysis system would not separate these residues. The low value probably reflects the fact that a portion of the cyanogen bromide cleavage product of methionine is present as basic homoserine lactone. ^c Yields are based on micromoles of peptides isolated compared with micromoles of fragment originally digested with trypsin.

Carboxypeptidase A and B digestions (2% w/w) were carried out in 0.1 ml of 0.2 M ammonium bicarbonate for 2–18 hr at 37°.

Cyanogen Bromide Cleavage and Fragment Separation. This cleavage was carried out on native M321 as described by Press *et al.* (1966). Cyanogen bromide fragments were separated on G-75 Sephadex (2.5×200 cm) in 4 M guanidine-HCl and 2% formic acid. Desalting was achieved through G-10 Sephadex (6.5×45 cm) in 1 M acetic acid.

Peptide Isolation. All peptides, apart from the cyanogen bromide fragments, were isolated from paper. Peptide maps were prepared as described by Katz *et al.* (1959). Peptides were eluted as described by Bennett (1967).

Dansyl-Edman Peptide Degradation. The dansyl-Edman procedure of Gray (1967) was followed. Dansyl amino acids were identified by thin-layer chromatography on polyamide sheets (Woods and Wang, 1971; Crowshaw *et al.*, 1967) as modified by Kaplan and Metzger (1969).

Half-cystine and tryptophan could not be identified by thin-layer chromatography. Generally small peptides were obtained with just a single tryptophan or 1/2-cystine residue and these residues were identified by amino acid analysis. All of the positions which gave blank dansyl end groups had tryptophan or 1/2-cystine residues that were homologous with M70, a similar mouse κ chain (Gray *et al.*, 1967).

Edman Sequenator Analysis. This procedure was carried out as described by Hood *et al.* (1973).

Amide Determinations. Amide determinations were carried out with carboxypeptidase A and B digestions followed by quantitative amino acid analysis.

TABLE III: Amino Acid Sequence of Tryptic Peptides from CN-C.

C-T2	Ala-Thr-Ile-Ser(Cys,Arg)
C-T3	Ala-Ser-Lys
C-T4 ^a	Ser-Val-Asx-Thr-Tyr-Gly-Asx-Ser-Phe-Hsr
C-T19	Ser-Ser-Thr-Leu-Thr-Leu-Thr-Lys
C-T20	Asx-Glx-Tyr-Glx-Arg
C-T21 ^a	His-Asx-Ser-Tyr-Thr-Cys-Glx-Ala-Thr-His-Lys
C-T22	Thr-Ser-Thr-Ser-Pro-Ile-Val-Lys
C-T23	Ser-Phe-Asn-Arg
C-T24	Asn-Glu-Cys

^a The last two residues of these peptides were determined by carboxypeptidase A and B digestion followed by quantitative amino acid analysis.

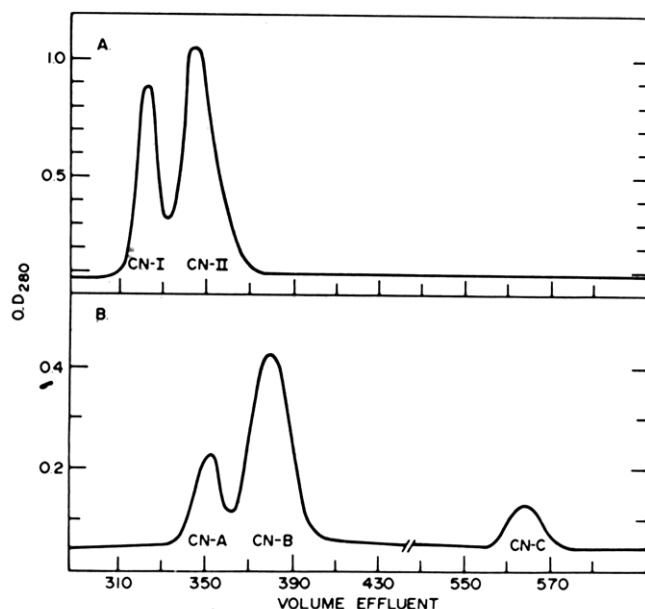


FIGURE 3: (A) Native M321 cleaved with cyanogen bromide and fractionated on G-75 Sephadex (see Materials and Methods and text). (B) Peak I or II (from part A) after reduction and alkylation and separation on G-75 Sephadex (see text).

Peptide Nomenclature. A prefix is assigned to each peptide designating the cyanogen bromide fraction from which the peptide was derived (see Figure 2, CN-B or CN-C). All peptides were numbered from the amino terminus and the cyanogen bromide designations were followed by a T designating trypsin,

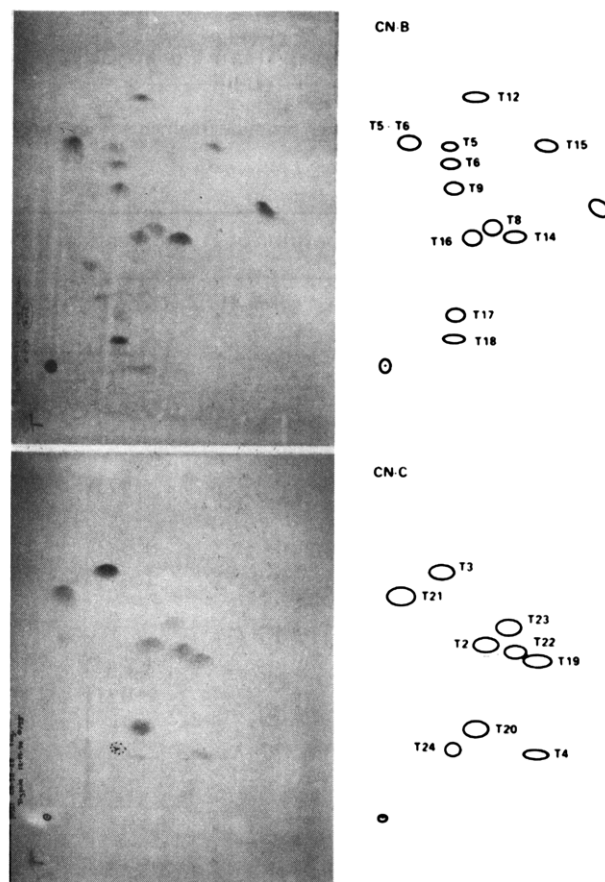


FIGURE 4: Tryptic fingerprints of cyanogen bromide fragments CN-B and CN-C. On the left is a photograph of the fingerprint and on the right a tracing of the respective peptides with numbering according to Figure 5 and Tables II, III, VI, and VII.

TABLE IV: Amino Acid Composition of Chymotryptic Peptides from CN-C.^a

	C-C2	C-C3 ^d	C-C4 ^d	C-C5
Tyr			1	
Phe				0.9 (1)
Lys			1	
His				
Arg		1	1	
Asp			1	0.9 (1)
Glu		1		
Thr		1	1	
Ser	1.0 (1)		3	1.4 (2) ^b
Pro				
Ala	1.0 (1)	1	1	
Gly		1		0.8 (1)
Val	1.0 (1)		1	
Met				
CMCys			1	
Ile		1		
Leu	1.0 (1)			
Trp				
Total residues	4	6	11	5
Yield (%) ^c	32			49

^{a-c} See legend to Table II. ^d The composition of these peptides was determined qualitatively by high-voltage electrophoresis on paper.

a C for chymotrypsin and a TH for thermolysin. Residue numbering is according to Gray *et al.* (1967).

Results

Location of Methionyl Residues. Amino acid analysis of M321 gave 2 mol of methionine/mol of protein (Table I). One of the methionine residues is located at position 175 in the invariant C region of BALB/c κ chains (Gray *et al.*, 1967; Milstein and Svasti, 1971). The second was assigned to residue 37 after automatic sequenator analysis was carried out for 37 residues on the intact light chain. Thus the three methionine cleavage fragments were 37, 142, and 39 residues in size and, as shown in Figure 2, will be designated L-1, L-2, and L-3.

TABLE V: Amino Acid Sequence of Chymotryptic Peptides from CN-C.

C-C2	(Ala,Val,Ser,Leu)
C-C3	(Gly,Gln,Arg,Ala,Thr,Ile)
C-C4	(Ser,Cys,Arg,Ala,Ser,Lys,Ser,Val,Asx,Thr,Tyr)
C-C5 ^a	Gly-Asx-Ser-Phe-Hsr

^a The last three residues of this peptide were determined by carboxypeptidase A (see Legend to Table III).

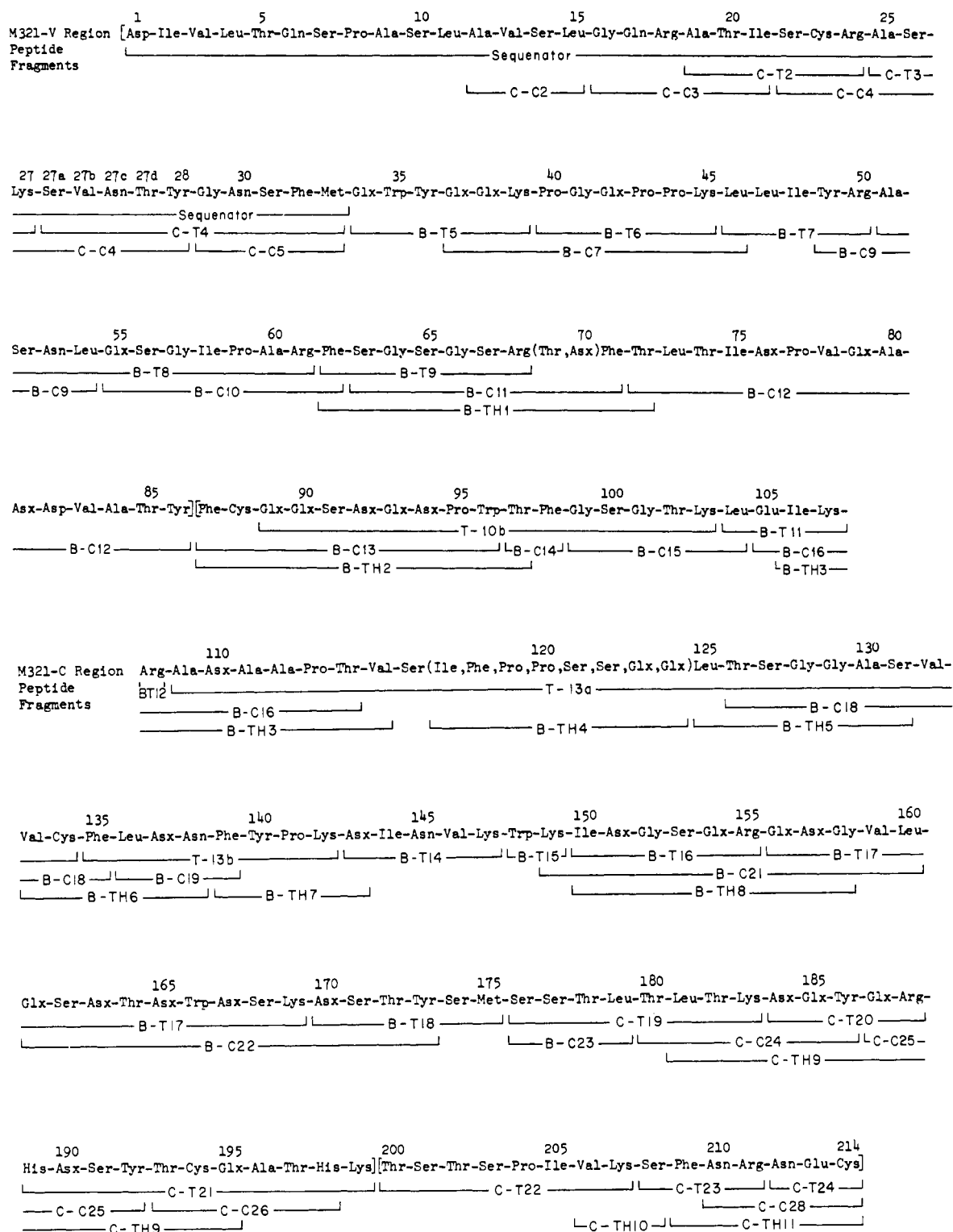


FIGURE 5: A summary of the information concerning the amino acid sequence of M321. Abbreviations are discussed in the text. The numbering is that of Gray *et al.* (1967).

Note that throughout these papers we will use the numbering of Gray *et al.* (1967) which is based on the sequence of M41. The κ chains in this group of light chains have an insertion of four residues at or near position 27 when compared to M41. Thus, for example, the methionine at residue 37 is assigned to position 33 by the numbering of Gray *et al.* (1967).

Isolation of Cyanogen Bromide Peptides. Native M321, cleaved with cyanogen bromide, gave the elution pattern on

G-75 Sephadex shown in Figure 3A. Peak I eluted at the position of light-chain aggregates and peak II at the position of light chain monomer. Tryptic peptide maps of peaks I and II were indistinguishable from one another and similar to those of intact light chain. Since one disulfide bridge spans the methionine at position 33 and a second disulfide bridge spans the methionine position 175, peak II represents the three cyanogen bromide fragments covalently linked through

TABLE VI: Amino Acid Composition of Tryptic Peptides from CN-B.^a

	B-T5	B-T6	B-T5,6	B-T7	B-T8	B-T9	B-T11	BT-12	B-T14	B-T15	B-T16	B-T17	B-T18
Tyr	0.8 (1)		0.7 (1)	0.8 (1)		0.8 (1)							0.9 (1)
Phe	1.2 (1)	1.0 (1)	1.8 (2)				0.9 (1)		1.0 (1)	1.0 (1)		1.0 (1)	
Lys													
His													
Arg				1.0 (1)	0.8 (1)	1.0 (1)		1.0 (1)			0.8 (1)		1.1 (1)
Asp					0.9 (1)				2.1 (2)		1.1 (1)	3.6 (4)	1.1 (1)
Glu	3.1 (3)	1.1 (1)	4.2 (4)		1.0 (1)		1.2 (1)				1.0 (1)	1.9 (2)	1.0 (1)
Thr												1.1 (1)	2.4 (3) ^b
Ser					1.9 (2)	3.3 (3)							
Pro		3.0 (3)	3.1 (3)		1.1 (1)								
Ala					2.0 (2)								
Gly		0.9 (1)	1.2 (1)		1.1 (1)	2.0 (2)					0.9 (1)	1.1 (1)	
Val								1.0 (1)				0.9 (1)	
Met													
CMCys													
Ile				1.2 (1)	1.0 (1)		1.0 (1)		1.0 (1)		1.2 (1)		
Leu				2.1 (2)	1.0 (1)		0.9 (1)					1.0 (1)	
Trp	0.9 (1)		0.6 (1)										
Total residues	6	6	12	5	11	7	4	1	5	2	6	14	6
Yield (%) ^c	4	13	7	11	31	36	29	15	34	32	64	6	29

^{a-c} See legend to Table II. ^d Tryptophan determined by Ehrlich's stain.TABLE VII: Amino Acid Composition of Chymotryptic and Thermolytic Peptides from CN-B.^a

	B-C7 ^d	B-C9	B-C10	B-C9,10	B-C11	B-C12	B-C13	B-C14	B-C15	B-C16	B-TH1 ^d	B-TH2
Tyr												
Phe	1.8 (2)											
Lys		0.9 (1)	1.0 (1)	0.9 (1)	1.0 (1)	0.9 (1)	0.9 (1)	1.1 (1)	1.0 (1)	0.8 (1)	1.9 (2)	1.0 (1)
His												
Arg		0.9 (1)	1.1 (1)	2.1 (1)	1.0 (1)	3.3 (3)	2.2 (2)			0.9 (1)	1.0 (1)	2.0 (2)
Asp		1.1 (1)		1.0 (1)	1.1 (1)	1.1 (1)	2.9 (3)			1.0 (1)	0.9 (1)	3.2 (3)
Glu	3.2 (3)		1.0 (1)	1.0 (1)		3.3 (3)		1.0 (1)	1.1 (1)	1.0 (1)	1.9 (2)	1.0 (1)
Thr					1.0 (1)		1.0 (1)		0.9 (1)		3.1 (3)	1.0 (1)
Ser		0.9 (1)	1.1 (1)	1.9 (2)	2.9 (3)	1.3 (1)	1.0 (1)			1.0 (1)		0.9 (1)
Pro	3.0 (3)		1.1 (1)	1.1 (1)		2.3 (2)	1.1 (1)			1.0 (1)		
Ala		1.1 (1)	1.0 (1)	2.2 (2)						3.1 (3)		
Gly			1.0 (1)		2.0 (2)				1.9 (2)			
Val	0.9 (1)			1.1 (1)		1.9 (2)						

TABLE X: Amino Acid Sequence of Tryptic Peptides from CN-B.

B-T5	(Glx,Trp,Tyr,Glx,Glx)Lys
B-T6	(Pro,Gly,Glx,Pro,Pro)Lys
B-T5,6	(Glx,Trp,Tyr,Glx,Glx,Lys,Pro,Gly,Glx,Pro,Pro,)Lys
B-T7 ^a	Leu-Leu-Ile-Tyr-Arg
B-T8 ^a	Ala-Ser-Asn-Leu-Glx-Ser-Gly-Ile-Pro-Ala-Arg
B-T9 ^a	Phe-Ser-Gly-Ser-Gly-Ser-Arg
B-T11 ^a	Leu-Glu-Ile-Lys
B-T12	Arg
B-T14 ^b	Asx-Ile-Asn-Val-Lys
B-T15 ^c	Trp-Lys
B-T16	Ile-Asx-Gly-Ser-Glx-Arg
B-T17	Glx-Asx-Gly-Val-Leu-Glx-Ser-Asx-Thr-Asx-Trp-Asx-Ser-Lys
B-T18	Asx-Ser-Thr-Tyr-Ser-Met

^a The C terminal basic residue was confirmed with carboxypeptidase B. ^b The last three residues were obtained from a carboxypeptidase A and B digest which permitted the amide assignment. ^c Tryptophan was determined by Ehrlich's stain and lysine was assumed to be C terminal.

TABLE XI: Amino Acid Sequence of Chymotryptic and Thermolytic Peptides from CN-B.

B-C7	Glx-Glx-Lys-Pro-Gly-Glx-Pro-Pro-Lys-Leu
B-C9 ^a	(Tyr,Arg,Ala,Ser)Asn-Leu
B-C10	Glx(Ser,Gly,Ile,Pro,Ala,Arg,Phe)
B-C11 ^a	Ser(Gly,Ser,Gly,Ser,Arg,Thr,Asx)Phe
B-C12 ^a	Thr-Leu-Thr-Ile-Asx-Pro-Val-Glx-Ala-Asx-Asp-Val-Ala-Thr-Tyr
B-C13	Phe-Cys-Glx-Glx-Ser-Asx-Glx-Asx-Pro-Trp
B-C14	Thr-Phe
B-C15	Gly-Ser-Gly-Thr-Lys-Leu
B-C16	Glu-Ile-Lys-Arg-Ala(Asx,Ala,Ala,Pro,Thr)
B-TH1	(Phe,Ser,Gly,Ser,Gly,Ser,Arg,Thr,Asx,Phe,Thr)
B-TH2	(Phe,Cys,Glx,Glx,Ser,Asx,Glx,Asx,Pro,Trp,Thr)

^a The C-terminal one to three residues were confirmed with the carboxypeptidase A procedure.

cystine bonds. Peak I is an aggregate of these monomers. Both peaks had amino-terminal aspartic acid and serine by dansyl analysis. The third expected amino-terminal residue was blocked (see below).

The disulfide bridges joining the cyanogen bromide fragments were cleaved by reduction and S-carboxymethylation. The elution patterns of both peaks I and II on Sephadex were essentially identical and are shown in Figure 3B. The first peak, CN-A, produced a tryptic fingerprint similar to intact M321 and was set aside as it probably represented aggregates of the fragments and/or incomplete cleavage products. The second peak, CN-B, and third, CN-C, gave restricted and nonidentical tryptic fingerprints (Figure 4). The yields of the CN-B and CN-C fragments were 55 and 80%, respectively.

Characterization of CN-C. Dansyl end-group analysis on the intact CN-C gave amino-terminal aspartic acid and serine residues. Carboxypeptidase A treatment gave homoserine and phenylalanine. Nine tryptic peptides were isolated from this fraction. Their amino acid compositions and yields are given in Table II and their amino acid sequences in Table III. These sequence data are summarized in Figure 5 and are consistent with the presence of fragments L-1 (residues 1-33) and L-3 (residues 176-214) in the CN-C fraction.

Some chymotryptic peptides were isolated from CN-C. Their compositions and amino acid sequences are given in Tables IV and V respectively. Since peptide C5 falls in a region which demonstrates considerable sequence diversity when different light chains are compared (a hypervariable region, see Wu and Kabat, 1970), this peptide was completely sequenced to give an independent check of this hypervariable region.

Characterization of CN-B. The amino terminus of this peptide was unreactive to dansyl chloride and phenyl isothiocyanate (manual and sequenator). Carboxypeptidase A released homoserine, serine, and some tyrosine from the C terminus. Thirteen tryptic peptides were isolated from fingerprints of CN-B. Their compositions are given in Table VI and their amino acid sequences in Table X. These data are summarized in Figure 5 and represent peptides from fragment L-2 (residues 34-175). Peptides T10 and T13 could not be recovered from the S-carboxymethylated CN-B fragment.

The tryptic cleavage between peptides T5 and T6 was incomplete and gave a T5,6 tryptic peptide as well as the individual fragments. This is consistent with the incomplete cleavage that is generally seen at this lysine-proline bond in other κ chains (L. Hood, unpublished result). Furthermore, T5 and

TABLE XII: Amino Acid Composition of Tryptic Peptides from Aminoethylated Light Chain.^{a,d}

	T10b	T13a	T13b	T21a ^e	T21b ^e
Tyr			1.0 (1)	1	
Phe	1.1 (1)	1.2 (1)	1.6 (2)		
Lys	1.0 (1)		1.0 (1)		1
His				1	1
Arg					
Asp	1.9 (2)	1.2 (1)	2.3 (3)	1	
Glu	3.2 (3)	2.3 (2)			1
Thr	2.0 (2)	2.1 (2)		1	1
Ser	1.9 (2)	5.2 (5)		1	
Pro	1.0 (1)	3.1 (3)	1.1 (1)		
Ala		3.8 (4)			1
Gly	2.0 (2)	2.3 (2)			
Val		2.9 (3)			
Met					
AECys ^f		1		1	
Ile		1.0 (1)			
Leu		1.1 (1)	1.0 (1)		
Trp	0.6 (1)				
Total residues	15	26	8	6	5
Yield (%) ^e	9	6	13		

^{a,c} See legend to Table II. ^d Other C region tryptic peptides had compositions in agreement with those in Tables II and VI (see text). ^e Determined qualitatively by high-voltage electrophoresis on paper. ^f Aminoethylcysteine (see text).

T5,6 were unreactive with dansyl chloride and phenyl isothiocyanate as was the intact CN-B fragment indicating that the probable glutamine residue at position 35 had cyclized to a pyrrolidonecarboxylic acid—possibly during the acidic conditions of cyanogen bromide cleavage.

Selected chymotryptic and thermolytic peptides were isolated from the variable portion of CN-B. The compositions and sequences are given in Tables VII and XI, respectively. Chymotryptic and thermolysin peptides from the C region of CN-B and CN-C were identified by qualitative analysis on paper (Table VIII).

Automatic Sequence Analysis of the Amino Terminus of M321. The recovery data of hydrophobic residues for the first 37 steps are given in Figure 6 and the amino acid sequence is summarized in Table IX. These results were confirmed by amino acid analysis of the PTH derivatives after acid hydrolysis (see Hood *et al.*, 1973). Also given are the radioactive counts of the PTH derivatives surrounding position 23. These data establish that the [¹⁴C]carboxyamidomethylcystine residue is at position 23.

Peptides Isolated from Aminoethylated Light Chain. All of the C region peptides from a tryptic digest of aminoethylated M321 could be isolated except one. A number of variable region peptides are also present. A typical fingerprint and the location of individual C region peptides is given in Figure 7. The amino acid compositions and sequences of one variable region peptide isolated from such a fingerprint are given in Tables XII and XIII. Also given are the amino acid compositions and sequences of the peptides on either side of the tryptic splits at aminoethylcysteine residues at positions 134 and 194. The remainder of the C region peptides derived from aminoethylated M321 had compositions and amino-terminal end

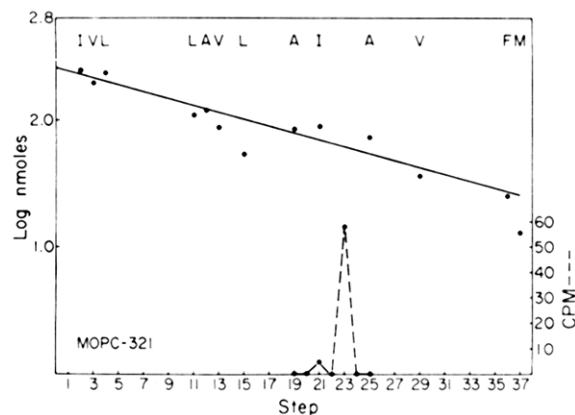


FIGURE 6: Yield data from automatic sequencer analysis of M321. These data were determined from gas chromatographic analysis of hydrophobic residues after correction for sample loss against an internal standard of norleucine (see preceding paper, Hood *et al.*, 1973). Dotted line indicates the counts in steps 21–25 due to [¹⁴C]-carboxyamidomethylcystine

groups in agreement with those derived from fragments CN-B and CN-C. The single C region peptide which could not be isolated by this procedure extends from positions 170 to 183. It can be readily isolated as tryptic peptides B-T18 and C-T19 from light chain cleaved with cyanogen bromide.

Amides. Amide residues are present at positions 53, 105, 138, 145, 210, and 212 as determined by carboxypeptidase treatment of appropriate peptides followed by quantitative amino acid analysis (Table XIV). Amides at positions 5, 17, and 27c were determined by thin-layer chromatography after automatic Edman degradation.

Ordering of Peptide Fragments. All of the sequence information is summarized in Figure 5. The order of the three cyanogen bromide fragments (Figure 2), L-1, L-2, and L-3, is unambiguous because L-1 has the same amino-terminal sequence as the intact light chain, L-2 has a C-terminal homoserine, and L-3 has the same C-terminal tryptic peptide as the intact protein. These assignments are also supported by extensive sequence homology with three mouse κ chains taken from the literature.

The order of tryptic and chymotryptic peptides in L-1 is completely determined by the sequencer analysis.

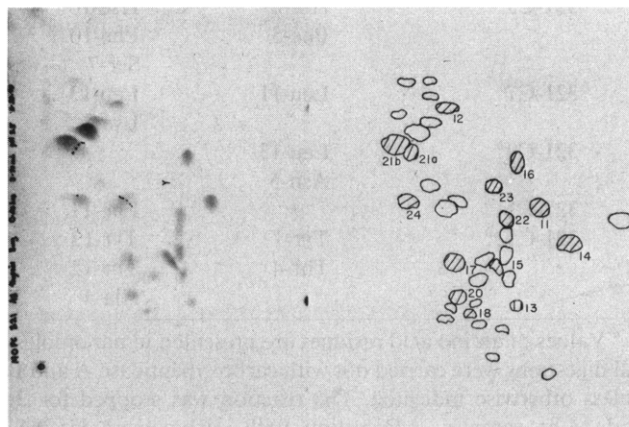


FIGURE 7: Tryptic fingerprint of aminoethylated M321. Photograph on left shows standards at the extreme right (arginine, alanine, and aspartic acid). The C region peptides are shaded and labeled according to Figure 5.

TABLE XIII: Amino Acid Sequence of Tryptic Peptides from Aminoethylated Light Chain.

T-10b	(Glx,Glx,Ser,Asx,Glx,Asx,Pro,Trp,Thr,Phe,Gly,Ser,Gly,Thr)Lys
T-13a	Ala-Asx-Ala-Ala-Pro-Thr(Val,Ser,Ile,Phe,Pro,Pro,Ser,Ser,Glx,Glx,Leu,Thr,Gly,Gly,Ala,Ser,Ser,Val,Val)Cys
T-13b	Phe-Leu-Asx-Asn-Phe-Tyr-Pro-Lys
T-21a	His(Asx,Ser,Tyr,Thr)Cys
T-21b	Glx(Ala,Thr,His)Lys

The peptides in L-2 are ordered in three groups, residues 34–87, 88–147, and 148–175 (Figure 5). Peptides in this last group overlap with C-terminal homoserine and accordingly must occupy the C-terminal position of L-2. The residue at position 34 is blocked to Edman degradation just as is the

intact L-2 fragment. Thus, residues assigned to positions 34–87 must comprise the amino-terminal portion of L-2. The middle group of peptides from 88 to 147 must by a process of elimination be in the middle portion of L-2.

The peptides of fragment L-3 can be ordered in two groups, 176–199 and 200–214 (Figure 5). The first group has an amino terminal serine, the same as the intact L-3 fragment, and accordingly must occupy the amino-terminal portion of L-3. In addition, the second group has the same C-terminal tryptic peptide as the intact M321.

These assignments are supported by homology comparisons with M70, M41 and M21 (Gray *et al.*, 1967; Milstein and Svasti, 1971).

Partial Sequence of V and C Regions. A summary of the partial amino acid sequence of M321 is given in Figure 5. The order of residues in the variable region is completely determined apart from residues at positions 69 and 70. In addition, we cannot rule out the possibility of an additional chymotryptic peptide between positions 86 and 87, although this possibility seems unlikely based on the homology of the V region of M321 with that of M70. The C region sequence is also completely determined apart from two stretches, residues 110–124 and 131–134.

Comments on the Use of Paper for Peptide Fractionation. The partial sequence of M321 was determined on approximately 900 mg of protein. Twenty-one of the expected 24 tryptic peptides were isolated from the three cyanogen bromide fragments after carboxymethylation. One peptide, T-1, gives low ninhydrin color yields and no serious attempt was made to isolate this peptide as the sequenator analysis had determined the sequence in this region. Two other peptides, T-10 and T-13, are large, 35 and 33 residues, respectively, and very hydrophobic. Such peptides tend to smear on paper and accordingly are recovered in very low yield. The regions of sequence including peptides T-10 and T-13 were obtained from chymotryptic fragments or tryptic fragments of aminoethylated light chain. It was often necessary to shorten the chromatography times for small and hydrophobic fragments and to increase the electrophoretic time to separate certain other peptide fragments.

The peptide yields from all the procedures ranged between 4 and 64%. Attempts to elute with solvents other than water (0.5 M ammonia–1.0 N acetic acid) did not seem to improve the yield of peptides.

Some difficulty was observed with charge heterogeneity of peptides. For example, T-17 was recovered in low yield from three different positions on the peptide map. We assume these differing mobilities were due to deaminations either in the mouse or during purification of the protein.

Discussion

The presence of two methionine residues in the light-chain M321 permits the separation of amino- and carboxy-terminal fragments L-1 and L-3 (37 and 39 residues respectively) from

TABLE XIV: Carboxypeptidase Digestion of M321 Peptides.^a

Peptide	3 hr	14 hr
321-T4 ^b	Hse-11 Phe-9	Hse-12 Phe-11 Ser-3
321-T7	Arg-7 Tyr-5	
321-T8	Arg-11	
321-T9	Arg-23 Ser-16	
321-T11	Lys-17 Ile-6	Lys-24 Ile-22 Glu-16 Leu-17
321-T13b ^{b,d}	Phe-6 Asn-4	
321-T14	Lys-14 Val-5	Lys-19 Val-19 Asn-5
321-T21	Lys-7 His-4	
321-T23	Arg-16 Asn-5	
321-T24		AECys-5 Glu-6 Asn-6
321-C5 ^b	Hse-8 Phe-5	Hse-10 Phe-10 Ser-7
321-C7 ^c	Leu-11	Leu-13 Lys-8
321-C9 ^b	Leu-13 Asn-5	
321-C11 ^b		Phe-11
321-C12 ^b	Tyr-7 Thr-4	Tyr-13 Thr-12 Ala-4

^a Values of amino acid residues are presented in nanomoles. All digestions were carried out with carboxypeptidase A and B unless otherwise indicated. The reaction was stopped for 3- and 14-hr samples. ^b Digestion with carboxypeptidase A. ^c The 3-hr sample was digested with carboxypeptidase A and the 14-hr sample with A and B. ^d Chymotryptic cleavage of tryptic peptide.

fragment L-2 (142 residues). Sequenator analysis on the intact protein provided the amino-terminal 37 residues. An analysis of peptide fragments from the cyanogen bromide pieces permitted us to order the residues in the V region and to confirm most of the C region sequence previously reported for other mouse κ chains (Gray *et al.*, 1967; Milstein and Svasti, 1971). M321 is identical in length and shows extensive amino acid sequence homology to a previously characterized mouse κ chain, M70 (Gray *et al.*, 1967). The extensive amino acid sequence homology between these two proteins supports our peptide alignments, particularly in the C region where no effort was made to obtain quantitative data.

The amino acid sequence of M321 with reference to M70 is also of interest because both share an insertion of four residues at or near position 27 when compared to mouse κ chains M41 and M21. The genetic and evolutionary implication of this observation will be discussed in the following paper (McKean *et al.*, 1973).

The C regions of M321 and M41 differ from that of the completely sequenced M21 by two residues, 161 and 165. This difference represents an interchange of an Asx and a Glx within a tryptic peptide and probably constitutes a sequence error. We have rechecked this region by dansyl-Edman analysis of the appropriate peptide and have reconfirmed the sequence given in Figure 5. Similarly, M321 and M21 differ from the published sequence of M41 at two positions, 127 and 129. Once again this difference probably represents an interchange (Gly-Ser). If any of these differences are substantiated, they might be explained by gene duplication, translational ambiguities, or multiple alleles at a single structural locus. This latter alternative seems particularly unattractive because of the highly inbred nature of the BALB/c mouse.

The paper techniques for isolating peptide fragments are rapid and simple and accordingly provide a useful approach for determining modest numbers of interesting κ -chain sequences. Using an approach similar to that described in this paper, two additional mouse κ chains with limited variations in the M321 group have been partly sequenced. The analysis of these chains and the genetic and evolutionary implications

which arise from the resulting sequence patterns are the subject of the following paper.

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