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Partial Sequences of Six Macroglobulin Light Chains. Absence of Sequence Correlates to Functional Activity*

Allen P. Kaplan† and Henry Metzger‡

ABSTRACT: The amino acid sequence of portions of the variable regions of the light chains from six Waldenström macroglobulins has been determined. Four of the six proteins studied show evidence of binding activity. No distinctive sequences which correlated with the functional properties of the active proteins were observed.

The data also strengthen previous evidence that light chains associated with μ-type heavy chains are not as a group distinguishable from those associated with other heavy-chain classes.

The polypeptide chains of immunoglobulins are divisible into regions having an invariant amino acid sequence (for chains of a particular class and allotype) and regions in which the sequence is variable (Cohen and Milstein, 1967). In light chains the variable region comprises about 100 amino acids, almost exactly the amino-terminal half of the chains. Although there is much less information on the heavy polypeptide chains the variable stretch may also be about 100 amino acids long (Gottlieb *et al.*, 1968), therefore making up approximately one-fourth the length of these chains.

These amino-terminal regions are the only ones which show sufficient variability to account for the remarkable binding specificity exhibited by functional immunoglobulins. Furthermore in at least one instance it has been possible to isolate a light-chain dipeptide from a site-labeled antibody preparation and this peptide was shown to have originated from the variable region (Singer and Thorpe, 1968).

Almost all of the published sequence data on immunoglobulins was derived from the polypeptide chains of myeloma proteins or Waldenström macroglobulins. None of these proteins had defined antibody activity. In the few instances where normal serum immunoglobulins were examined these were also without known binding activity (Hood *et al.*, 1966; Cohen and Milstein, 1967; Niall and Edman, 1967). In one instance the light chains of an antibody preparation have been studied (rabbit antidinitrophenyl antibody) but the data were limited and no unique sequence could be resolved (Doolittle, 1966). Other data available indicate that the N-terminal amino acids of the κ chains from several cold agglutinins (monoclonal human γM proteins which agglutinate human red cells in the cold) were similar to those seen with other κ chains (Cohen and Cooper, 1968). In addition, the sequence of the 22 amino acids at the N-terminal end of the κ chain from a cold agglutinin was recently published (Edman and Cooper, 1968).

The discovery in our laboratory of Waldenström macroglobulins with well-defined binding properties (Metzger, 1967;

* From the Arthritis and Rheumatism Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014. *Received May 28, 1969.*

† Present address: Robert Breck Brigham Hospital, Boston, Mass.
‡ To whom inquiries should be addressed.

Ashman and Metzger, 1969) prompted us to do a preliminary investigation of the sequence of these proteins. The availability of a simple method for isolating the amino-terminal peptide containing about 17% of the light-chain variable region (Hood, 1968) made examination of this region a useful initial approach. In the present work the amino-terminal sequences from six macroglobulin light chains are presented. Four of the six proteins possess some demonstrable binding activity (Table I) while two do not. Of the four which do, the activity of two of them is strongly reminiscent of *bona fide* antibody activity.

Materials and Methods

Proteins. Macroglobulins were isolated from plasmaphoresis specimens obtained from patients with Waldenström's macroglobulinemia. Plasma containing γM_{Ioe}, γM_{Wag}, γM_{Koh}, and γM_{How} were gifts from Drs. H. Fahey (National Institutes of Health), W. Terry (National Institutes of Health), J. Johnson (National Institutes of Health), and C. E. Bukley, III (Duke University), respectively.

Enzymes. Trypsin treated with L-(tosylamido-2-phenyl)-ethyl chloromethyl ketone and chymotrypsin were purchased from Worthington Biochemical Corp. and stored as 1% solutions in 0.001 N HCl at −20°. Carboxypeptidase A treated with diisopropyl fluorophosphate was a Worthington product and was stored as a 1% solution in 2.0 M $NaHCO_3$ at −80° in sealed tubes. Carboxypeptidase B, treated with diisopropyl fluorophosphate, was also a Worthington product (6.1 mg/ml) and was stored at −20°.

Subtilisin was purchased from Nutritional Biochemical Corp. and was stored as a 1% solution in 0.001 N HCl at −80°.

Pyrrolidonyl peptidase (Doolittle and Armentrout, 1968) was a gift from Dr. Leroy Hood.

Dowex. Dowex cation-exchange resin AG-50W-X2 (100–200) mesh was obtained from Bio-Rad Laboratories and used in the H^+ form by washing successively with 2 N NaOH, water, 2 N HCl, and then water until the pH of the effluent was neutral.

Edman Reagents. Pyridine, *N,N*-dimethylallylamine, benzene, butyl acetate, ethyl acetate, and dichloroethane were obtained reagent grade and redistilled prior to use. Reagents were checked periodically for the presence of peroxides by adding 20 μl of a 1.0% starch solution and 20 μl of 1.0% potassium iodide to 0.5 ml of reagent. Reagents were distilled when a positive test (blue color after 1 hr at room temperature) was obtained.

Phenyl isothiocyanate purchased from Eastman Chemical Co. was redistilled *in vacuo* and stored at −20° in sealed vials.

PTH[1]-amino acids were purchased from Mann Research Laboratories. Eastman silica gel chromatogram sheets (K301R) with fluorescent indicator were used for thin-layer chromatography of PTH-amino acid derivatives.

Dansyl-Cl and dansylamino acids were purchased from Pierce Chemical Co. Polyamide thin-layer chromatography sheets were obtained from Chiang Chin Trading Co., Ltd. (Gallard-Schlesinger, Chemical Manufacturing Co.).

Other Reagents. Dithiothreitol was purchased from Cali-

[1] Abbreviations used are: PTH, phenylthiohydantoin derivative; dansyl, 1-dimethylaminonaphthalene-5-sulfonyl.

TABLE I: Sources of Light Chains.

Light Chain	Protein	Activity	Comments	References
κ_{Lay}	γM_{Lay}	Anti-human γG	Activity intrinsic to protein	Metzger (1967)
			Activity solely in Fab fragments	
			One combining site per heavy–light-chain pair	
			Highly specific for human γG	
κ_{Wag}	γM_{Wag}	Anti-nitrophenyl derivatives	Activity intrinsic to protein	Ashman and Metzger (1969)
			Activity solely in Fab fragments	
			One combining site per heavy–light-chain pair	
			Highly specific, binding *p*-nitrophenyl derivatives preferentially	
κ_{Mar}	γM_{Mar}	Prevents activation of tissue lipoprotein lipase; ?anti-heparin	Inhibition of epididymal lipase in four experiments	A. P. Kaplan and C. S. Glueck, unpublished observations
κ_{Ioe}	γM_{Ioe}	None known	No detectable activity *vs.* nitrophenyl ligands. Same protein whose μ chain sequence has been published by Wikler *et al.* (1969)	
κ_{How}	γM_{How}	None known	No detectable activity *vs.* nitrophenyl ligands	
λ_{Koh}	γM_{Koh}	Anti-human γG	Activity intrinsic to protein. Subunits fail to inhibit precipitation. Highly specific for human γG	J. Johnson, unpublished observations

fornia Biochemical Corp. and was sublimated prior to use. Iodoacetamide was recrystallized twice from water. Hydrazine was purchased from Matheson Coleman and Bell and was redistilled *in vacuo* over $CaCl_2$ and stored in 0.5-ml aliquots in sealed vials at −80°.

Isolation of Macroglobulins. The macroglobulins γM_{Wag}, γM_{Mar}, and γM_{Ioc} were isolated by repeated euglobulin precipitation. Proteins γM_{Lay} and γM_{Koh} react with human γG to give complexes which precipitate in the cold. The precipitates were freed of the γG components using ultracentrifugation in 1 M KSCN followed by DEAE-cellulose chromatography as described previously (Stone and Metzger, 1969). Protein γM_{How} was precipitated from 40% saturated ammonium sulfate and chromatographed on DEAE-cellulose and then on G-200 Sephadex. The material eluting with the void volume was free of γG and of other serum contaminants.

Isolation of Heavy and Light Chains. The macroglobulins were mildly reduced with dithiothreitol and alkylated with iodoacetamide as previously described (Miller and Metzger, 1965). The heavy and light chains were separated on a G-100 Sephadex column equilibrated with 1 M propionic acid and lyophilized.

Performic Acid Oxidation. In preparation for Edman degradation, a portion of each light chain was performic acid oxidized (Hirs, 1967).

Acetylation of κ Chains. Those light chains with free amino-terminal groups (from γM_{Wag}, γM_{Ioc}, γM_{Mar}, γM_{How}, and γM_{Lay}) were acetylated (Hood, 1968): 50 mg of protein was dissolved in 2 ml of freshly deionized 10 M urea and 3 ml of redistilled pyridine was added slowly. The solution was cooled at 4° and three 0.1-ml portions of acetic anhydride were added at 5 min intervals with constant stirring. The solution was dialyzed against 0.2 M NH_4HCO_3 for 12 hr at 4° and then against 10 M urea–1.0 M Tris-HCl (pH 8.6) for 6 hr at room temperature.

Reduction of Intrachain Disulfide Bonds. The dialyzed solutions of acetylated light chains were made 0.05 M in dithiothreitol by addition of solid reagent and incubated for 1 hr at 37°. A 10% molar excess of iodoacetamide was added, and the solution was incubated for 15 min at room temperature, and then dialyzed against repeated changes of 0.1 M NH_4HCO_3.

Aminoethylation of λ Chain. The λ light chain of γM_{Koh} has a blocked amino-terminal residue and therefore did not require acetylation. The λ chains frequently have no basic residue proximal to cysteine at position 22. In order to make the peptide bond carboxy terminal to this position sensitive to tryptic attack the cysteine was aminoethylated (Cole, 1967): 50 mg of this light chain was dissolved in 2.5 ml of 0.2 M Tris-HCl (pH 8.6) containing 10 M urea. To this was added 120 mg of recrystallized β-mercaptoethylamine hydrochloride in 1 ml of water. After reduction for 1.5 hr at room temperature under nitrogen, 280 μl of ethylenimine was added three times at 20-min intervals with constant stirring. Excess ethylenimine was quenched with 1 ml of β-mercaptoethanol and the solution was then dialyzed against repeated changes of 0.1 M ammonium bicarbonate.

Isolation of N-Terminal Tryptic Peptides. The N-terminal tryptic peptide from the light chains was isolated according to the following principle (Hood, 1968). The chains were digested with trypsin, the newly exposed carboxy-terminal basic residues were removed with carboxypeptidase B, and the mixture of peptides was applied to a cationic exchange resin. Since only the terminal peptide with a blocked amino group lacks a positive charge it will be preferentially eluted, all other peptides being more or less firmly bound by the resin. Tryptic digestion was carried out at 37° for 4 hr in 0.1 M NH_4HCO_3 using 2% w/w of the enzyme. Carboxypeptidase B (2% w/w) was then added and the solution was incubated for another hour and then lyophilized. The peptides were dissolved in 5 ml of water and applied to a 15 × 1 cm Dowex 50 column equilibrated with water. The column was eluted at a rate of 20 ml/hr and 1.2-ml fractions were collected. The optical density at 215 mμ was determined using a Zeiss PMQ 11 spectrophotometer.

Amino Acid Analysis. Samples were lyophilized, dissolved in 0.1 ml of 6 M HCl, repeatedly evacuated and flushed with water-purified N_2, sealed under vacuum, and incubated at 105° for varying lengths of time. The seal was broken, the HCl was removed under vacuum in a desiccator, and the residue was dissolved in 0.4 ml of 0.02 M HCl. Analyses were performed on a Beckman Model 120C automatic amino acid analyzer. Serine and threonine contents were determined by extrapolating the values obtained after 8, 16, and 24 hr of hydrolysis to zero time. In a few instances a 46 × 20 cm sheet of Whatman chromatography paper was spotted with the sample and appropriate standards. The paper was wet with 7.4% formic acid (pH 1.64) and electrophoresed at 38° for 9000 V hr (4500 V for 2 hr) using a Gilson Model D electrophorator containing 7.4% formic acid. Under these conditions only serine-valine and threonine-proline are unresolved.

Hydrazinolysis. To 10–15 nmoles of lyophilized light chain or peptide was added 0.05 ml of anhydrous hydrazine. The tube was flushed with N_2, sealed, and incubated at 70° for 48 hr. The seals were broken and the contents were dried under vacuum in a desiccator in the presence of NaOH and P_2O_5. The residue was dissolved in 0.4 ml of 0.02 M HCl and the carboxy-terminal residue was determined either on the amino acid analyzer or by high-voltage paper electrophoresis.

Carboxypeptidase A Digestion. To 15 nmoles of lyophilized peptide was added 0.1 ml of 0.1 M NH_4HCO_3 and approximately 2% w/w carboxypeptidase A. The reaction was allowed to proceed for 24 hr at room temperature. A control tube containing no peptide was run simultaneously in order to quantitate the release of amino acids due to autodigestion of the enzyme. Identification of released amino acids was performed on the amino acid analyzer.

Isolation of Subtilisin Peptides. The lyophilized N-terminal tryptic peptide (250–300 nmoles) was dissolved in 1–2 ml of 0.1 M NH_4HCO_3 and 2% w/w subtilisin was added. After a 4-hr incubation at 37° the solution was lyophilized, dissolved in 0.4 ml of water, and spotted on Whatman 3MM chromatography paper. Descending chromatography using a butanol–acetic acid–water solvent was followed by electrophoresis in a pH 3.5 pyridine–acetic acid buffer in the standard manner (Bennet, 1967). The dried paper was sprayed with 0.05% ninhydrin in ethanol–2 N acetic acid (3:1, v/v) solution. Peptide spots appearing after successive sprayings were excised and eluted with 0.5 ml of water.

Edman Degradation. Performic acid oxidized light chains (0.5 μmole) were subjected to sequential amino-terminal (Edman) degradation using a modified three-cycle form of the phenyl isothiocyanate procedure as described by Doolittle *et al.* (1967). The PTH derivatives were identified by thin-layer silica gel chromatography. The sheets were dipped in 75

TABLE II: Amino Acid Composition of N-Terminal Tryptic Peptides.[a]

	Wag	Ioc	Lay	Mar	How	Koh
Arg	0	0	0	0	0	0
His	0	0	0	0	0	0
Lys	0	0	0	0	0	0
CM-Cys	0	0	0	0	0	0
Asp	2.04 (2)	1.80 (2)	1.92 (2)	1.85 (2)	0.18 (0)	0.14 (0)
Thr	0.97 (1)	0.86 (1)	0.97 (1)	1.01 (1)	1.60 (2)	3.11 (3)
Ser	4.40[b] (4)[c]	4.65[b] (5)	4.60[b] (5)	1.98[d] (2)	2.25[b] (2)[e]	3.75[b] (4)[f]
Glu	2.17 (2)	2.09 (2)	2.07 (2)	2.23 (2)	2.90 (3)	3.06 (3)
Pro	0.86 (1)	0.80 (1)	1.00 (1)	0.92 (1)	1.50 (2)	2.68 (3)
Gly	1.22 (1)	1.19 (1)	1.06 (1)	0.65 (1)	1.96 (2)	1.94 (2)
Ala	0.93 (1)	0.94 (1)	0.05 (0)	0.71 (1)	0.12 (0)	1.02 (1)
Val	1.15 (1)	0.94 (1)	2.00 (2)	0.66 (1)	1.04 (1)	1.86 (2)
Met	0.88 (1)	0.91 (1)	0.93 (1)	0. (0)	0 (0)	0 (0)
Ile	0.93 (1)	0.98 (1)	0.93 (1)	1.09 (1)	0.95 (1)	0.92 (1)
Leu	0.84 (1)	1.09 (1)	1.01 (1)	1.74 (2)	2.80 (3)	1.00 (1)
Tyr	0	0	0	0	0	0
Phe	0	0	0	0	0	0
Total	16[c]	17	17	14+	16[e]	20[f]

[a] Except where indicated results are for 24-hr hydrolyses. Values in parentheses are nearest whole numbers. [b] Extrapolated value. [c] Sequence analysis indicated 5 serines and therefore 17 residues. [d] Unextrapolated value. [e] Sequence analysis indicated 3 serines and therefore 17 residues. [f] Sequence analysis indicated 5 serines and therefore 21 residues.

ml of acetone–formamide mixture (10:3, v/v) and air dried prior to application of samples. Yields could be calculated by direct spectral analysis of the PTH derivative as long as the ratio of absorbancy at 245 to 269 mμ approximated 0.45 (Sjoquist, 1957). This usually pertained only for the first several residues, and 80–90% yields were calculated. On subsequent steps the ratio deteriorated so that the 269-mμ absorbance could no longer be assumed to represent only the PTH derivative. Even so, several further steps yielded qualitatively unambiguous results on thin-layer chromatography.

Methionine sulfone was determined by hydrolyzing the PTH derivatives in 6 M HCl for 24 hr at 150° (Van Orden and Carpenter, 1964). The tubes were evacuated and flushed with N_2 repeatedly before being sealed for hydrolysis. The hydrolysates were analyzed on the amino acid analyzer. Leucine and isoleucine were similarly determined because of the difficulty in distinguishing them on chromatography. Isoleucine undergoes 50% conversion into alloisoleucine under these conditions. Serine and threonine were determined by dansylation of the thiazolinone derivative as follows (Hood, 1968). A portion of the latter was hydrolyzed in 0.03 ml of 0.1 M NaOH at 105° for 12 hr in a sealed tube. The seal was broken, the tube was exposed to a Dry-Ice box atmosphere for 3 min (which lowers the pH to 8), and 0.03 ml of a 3 mg/ml of dansyl-Cl in acetone solution was added. After incubation at 40° for 90 min acetone was removed with an N_2 stream. The remaining solution was extracted twice with 0.06 ml of water-saturated ethyl acetate. The solution was then acidified to pH 4 with 0.1 M HCl and extracted with 0.1 ml of ethyl acetate to remove the dansyl-amino acid.

Dansyl–Edman Degradation. Sequential amino-terminal degradation by the Edman procedure and identification of successively exposed amino-terminal amino acids using the dansyl-Cl method was performed as described by Gray (1967). Dansylamino acids were identified by chromatography on thin-layer polyamide sheets (Woods and Wang, 1967); 1.5% formic acid and benzene–acetic acid (9:1, v/v) were used for the first and second dimensions, respectively. A third chromatography in the same direction as the second and employing ethyl acetate–methanol–acetic acid (20:1:1, v/v) was used to resolve serine from threonine and aspartic from glutamic acid (Crowshaw *et al.*, 1967).

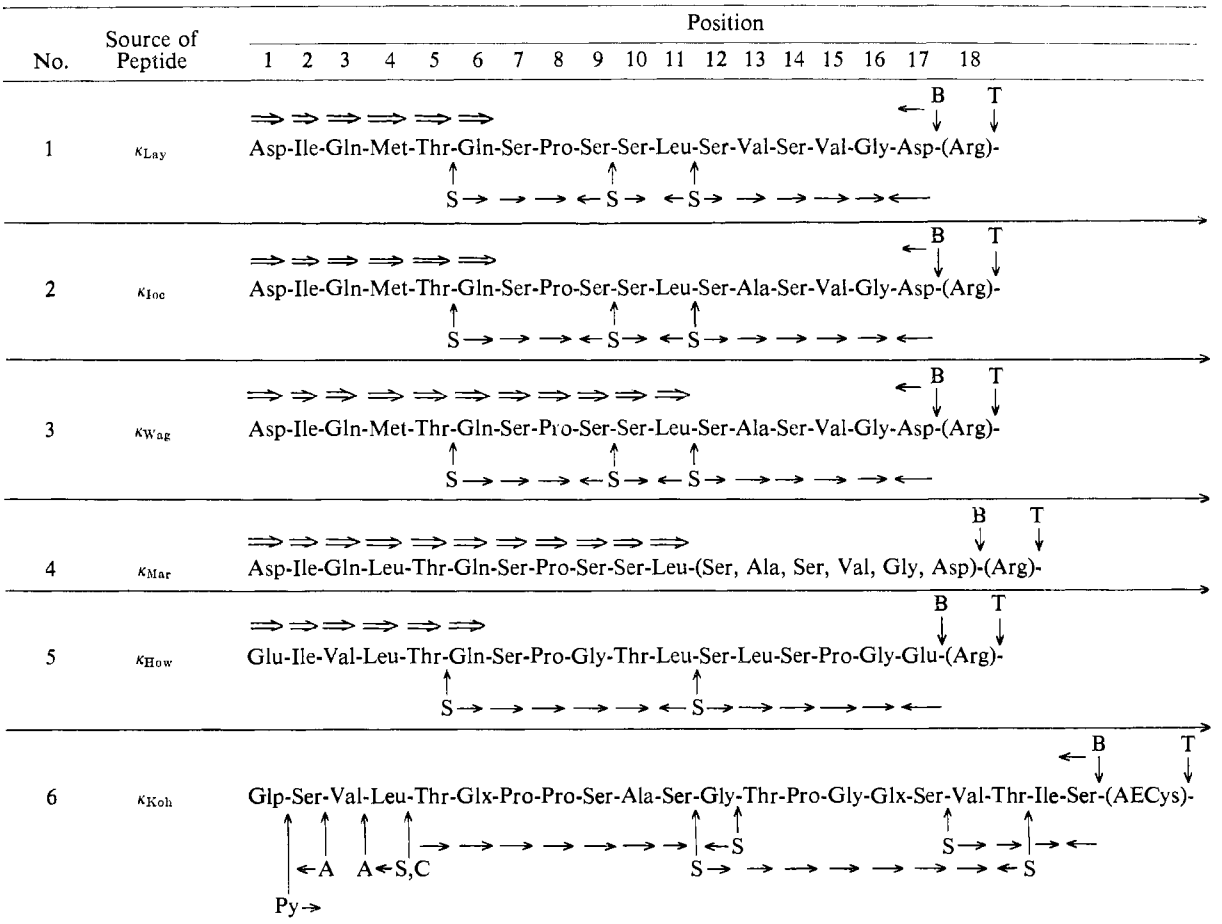
Pyrrolidonyl Peptidase Digestion. Lyophilized peptide (20 nmoles) was dissolved in 0.075 ml of 0.04 M sodium phosphate buffer (pH 7.3) containing 0.03 M mercaptoethanol and 0.001 M EDTA; 25 μl of purified enzyme was added and the solution was incubated for 16 hr at room temperature. This quantity of enzyme cleaved approximately one-half of a 2.5×10^{-2} M solution (25 μl) of the test substrate pyrrolidonylalanine when incubated at 30° for 1 hr. Ethanol (1 ml) was added to precipitate the protein and the newly exposed amino-terminal residue on the peptide in the supernatant was determined by hydrolysis of the dansylated peptide.

Results

Amino-Terminal Residues of Light Chains. Edman degradation of the light chains gave unambiguous results. Except for the λ light chain from γM_{Koh}, a single amino acid was readily identified at each position tested. The data are presented in Table III above each sequence.

Isolation and Characterization of Tryptic Amino-Terminal Peptide. No difficulty was encountered in isolating a sufficiently pure amino-terminal peptide from five of the six light chains. In eaeh case approximately 2 μmoles (50 mg) of light chains was treated with trypsin and carboxypeptidase B and

TABLE III: Summary of Procedures Used to Sequence N-Terminal Peptides.[a]



[a] Procedures performed on entire light chain are indicated above the sequence. Those performed on the isolated peptides are below the sequence. Key: ↑ or ↓, site of proteolytic attack; T, trypsin; B, carboxypeptidase B; A, carboxypeptidase A; Py, pyrrolidonyl peptidase; C, chymotrypsin; S, subtilisin; ⟹, Edman procedure with identification of PTH derivatives; →, Edman–Dansyl procedure; ←, hydrazinolysis. Arginine (in parentheses, sequences 1–5) is by analogy with other light chains. Aminoethylcysteine (AECys in parentheses, sequence 6) by analogy with other λ light chains. In sequence 6 separate peptides with overlapping sequences at positions 12 and 18–19 were isolated.

the mixture was then applied to a Dowex 50 column. Yields of the isolated peptides whose composition may be seen in Table II were approximately 11–21%.

We were not successful in isolating the amino-terminal peptide of K_{Mar} in preparative amounts. It was only possible to isolate enough for an amino acid composition. On repeated attempts not only was the peptide contaminated with other peptides which eluted with it from the Dowex column, but also the absolute yield of the amino-terminal peptide was low. We have no explanation for the reduced yield of this peptide.

Sequence Determinations. A summary of the procedures used to determine the sequence of amino acids for each of the N-terminal tryptic peptides is presented in Table III. In some instances additional subtilisin peptides were isolated which served to confirm the sequences, but did not add any information otherwise. These have been omitted from the table. More complete data on the peptides obtained from K_{Ioc} are presented in Table IV which also gives the yields of each of the subtilisin peptides. The data are representative of those obtained for the other peptides. All of the peptides are ordered unambiguously on the basis of the N- and C-terminal amino acids of the tryptic peptide as well as by overlaps.

The Ser-Leu peptide (peptide II in Table IV) is positioned only by exclusion in κ_{Lay} and κ_{Ioc}. In κ_{Wag} the ordering of this peptide was confirmed by Edman degradation of the κ chain (see Table III).

The sequence of the N-terminal four amino acids of λ_{Koh} were determined as follows. A portion of the subtilisin digest was applied to a Dowex 50 column. Only a blocked tetrapeptide was eluted. Hydrazinolysis gave the C-terminal Leu; 24 hr of carboxypeptidase A digestion gave equimolar amounts of valine and leucine from one aliquot and when another aliquot was then subjected to hydrazinolysis serine, valine, and leucine were obtained. The tetrapeptide was also digested with pyrrolidonyl peptidase and than dansylated. The dansylserine derivative was obtained, confirming the position of the serine

TABLE IV: Analysis of Ioc.

Amino Acid	Tryptic Peptide	Subtilisin Peptides I	II	III	IV	V	VI
Asp	1.80	0.12	0.09	0.06	0.25	*1.01*	*1.00*
Thr	0.86	*1.00*	0.08	0.02	0.11	0	0.05
Ser	4.65	0.23	*1.72*	*0.77*	*0.96*	*1.47*	*0.50*
Glu	2.09	0.12	*1.00*	0.06	0.10	0.07	0.25
Pro	0.80	0	*0.71*	0	0	0	0
Gly	1.19	0.13	0.16	0.03	0.28	*1.15*	*1.12*
Ala	0.94	0.12	0.08	0.03	*1.00*	*0.88*	0.11
Val	0.94	0.06	0	0	0	*0.95*	*0.87*
Met	0.91	*0.93*	0	0	0	0	0
Ile	0.98	0	0	0	0	0	0
Leu	1.09	0	0	*1.00*	0	0	0
Nanomoles recovered	250	110	75	118	25	83	45
Per cent recovery[a]	11	44	47	30	10	18	33
Light chain	Asp-Ile-Gln-Met-Thr-Gln-						
Tryptic peptide	(Asp-Ile-Gln-Met-Thr-Gln-Ser-Pro-Ser-Ser-Leu-Ser-Ala-Ser-Val-Gly-Asp)						
I	Met-Thr						
II	Gln-Ser-Pro-Ser						
Subtilisin peptides III	Ser-Leu-						
IV	Ser-Ala						
V	Ser-Ala-Ser-Val-Gly-Asp						
VI	Ser-Val-Gly-Asp						
Composite sequence	Asp-Ile-Gln-Met-Thr-Gln-Ser-Pro-Ser-Ser-Leu-Ser-Ala-Ser-Val-Gly-Asp						

[a] Yields of the tryptic peptides were calculated on the basis of the amount of light chain digested and for the subtilisin peptides of the basis of the amount of tryptic peptide digested.

and indirectly confirming the presence of N-terminal pyrrolidonecarboxylic acid. No overlap between the N-terminal peptide and the Pro_2,Thr,Glx,Ala,Ser peptide was obtained but on the basis of the composition this latter peptide must be adjacent to the N-terminal one.

Discussion

This study was undertaken to determine whether the polypeptide chains of Waldenström macroglobulins with specific binding activity had sufficiently distinctive N-terminal sequences to set them apart from the chains of myeloma proteins or Waldenström macroglobulins lacking that activity. As indicated in the introduction while the polypeptide chains of such proteins are similar to those of induced antibodies in their gross properties, little sequence data have been collected on functional immunoglobulins. We examined the amino-terminal regions of several light chains from such proteins. Although the segments examined comprise only 15–20% of the variable regions, past sequence data indicate that almost all of the positions in these segments show amino acid interchanges (Cohen and Milstein, 1967). Thus, if the binding activity of the proteins studied were related in some simple way to the linear sequence of the light polypeptide chains it was possible that study of even a limited portion of the variable region might be revealing.

No such correlation was found. In each case the light-chain sequence showed no gross difference from previously published sequence data. The data on γM_{Ioc} and γM_{Wag} are particularly striking since the light-chain sequences are identical for the first 17 amino acids even though γM_{Wag} clearly has combining sites different than those which might be present in γM_{Ioc} (Table I). Similarly the N-terminal 18 amino acids of κ chains of γM_{How} are identical with those of γM_{Ste} (Edman and Cooper, 1968) even though the latter γM is a cold agglutinin while the former is not. While the data in no way rule out the participation of the sequenced regions in the combining sites, their contribution must at a minimum be in conjunction with other variable segments. The possibility that the difference in binding activity of these proteins is totally related to the heavy (μ) chains cannot be ruled out, of course. On the other hand the sequence variability of the light chains of immunoglobulins as well as labeling (Metzger *et al.*, 1964; Singer and Thorpe, 1968; Metzger and Potter, 1968) and chain recombination (Porter and Weir, 1966) studies suggest an important contribution of the light chains to the combining sites.

We have performed an analysis of the N-terminal tetrapeptide released from the μ chains of each of the γM proteins described in this study (A. P. Kaplan, L. E. Hood, W. D. Terry, and H. Metzger, manuscript in preparation). Again no clear-cut correlation with the activities of the parent protein was found. For example, both μ_{Wag} and μ_{Koh} have the N-terminal sequence Gln-Val-Gln-Leu.

Our failure to find simple sequence correlates to the binding specificity exhibited by these proteins is not surprising. While

TABLE V: Comparison of N-Terminal Sequences from γM Light Chains and Light Chains from Other Sources.[a]

Prototype κ_I sequence	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
Non-γM variants (14 sequences)		Val, 0.07[b]	Leu 0.07	Leu, 0.14					Thr, 0.07	Thr, 0.21 Phe, 0.07					Leu, 0.07	Arg, 0.07		
γM variants (6 sequences)				Leu, 0.17						Thr, 0.17			Val, 0.33					
Prototype κ_{III} sequence	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	Gly	Glu	Arg
Non-γM variants (12 sequences)	Lys, 0.08 Asp, 0.08		Ile, 0.08	Met, 0.08					Ala, 0.17 Asx, 0.08								Asp, 0.17	
γM variants (4 sequences)		Met, 0.33		Met, 0.33					Ala, 0.67				Met, 0.33					
Prototype λ_I sequence	Glp	Ser	Val	Leu	Thr	Gln	Pro	Pro		Ser	Val	Ser	Gly	Ala	Pro	Gly	Gln	Arg
Non-γM variants (8 sequences)			Ala, 0.12								Ala, 0.25		Ala, 0.38	Thr, 0.38	Asx, 0.12			Thr, 0.12 Gly, 0.12 Lys, 0.12 Ala, 0.12
γM variants (1 sequence)											Ala, (1.0)			Thr, (1.0)				Ser, (1.0)

[a] Complete references for the entries in this table may be found in Hood and Talmage (1969). [b] The numbers under variable residues indicate the frequency with which they have been observed so far.

we felt it essential to rule out this possibility, it is more likely that the combining site configuration is the product of multiple (not necessarily consecutive) amino acids contributed by the tertiary folding of the polypeptide chains (Stryer, 1968; Cathou and Haber, 1967).

This study permitted us to examine an additional question. It has been shown from genetic data that the loci which code for the heavy and light chains of immunoglobulins segregate independently (Cohen and Milstein, 1967). It has also been shown that the light chains are grossly similar regardless of which class of heavy chain they were associated with in the completed immunoglobulin. That is, light chains derived from γG, γM, or γA immunoglobulins are similar in size, amino acid composition, electrophoretic dispersity, and antigenic structure. Only sequence analysis can, however, satisfactorily resolve the question as to whether certain kinds of light chains (differentiated with respect to their variable regions) are preferentially or exclusively associated with certain heavy-chain classes. Five γM light chains have previously been partially sequenced (Niall and Edman, 1967; Edman and Cooper, 1968). Two of the sequences fit into the κ_{I} subclass and three in the κ_{III} subclass of light chains (Hood *et al.*, 1967; Milstein, 1967). In two out of the four sequences variable residues were observed which had not been previously noted in non-γM light chains: Val-13 in γM_{Pap} and Met_2 and Met_{13} in γM_{Gra} (Table V). Our data add six more partial sequences of γM light chains. The data give additional support to the previous evidence that the light chains are not distinguishable on the basis of the class of heavy chains with which they were associated. There is only one position so far which is at all suspect: position 13 in κ_{I} at which Val has been found twice in 6 sequences of κ_{I} chains derived from γM proteins and never in 14 sequences of non-γM κ_{I} chains. The probability of such a distribution occurring by chance (0.07) is still considerable, however.

Variable regions of light chains within a particular subclass show a limited kind of variation (Hood and Talmage, 1969). Substitutions from subclass prototype sequences tend to be (1) limited to a few variants at any one position and (2) often identical to the predominant amino acid residue seen at that same position in the prototype sequence of another subclass. Our data adds four additional examples of this type of variation, Val_{13} (κ_{Lay}), Leu_4 (κ_{Mar}), Val_{10}, and Thr_{13} (λ_{Koh}).

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