conclude that the proportions of bond fission in the various positions are at C, 0.59–0.67, at D, 0.36–0.28, and at E + F, 0.053. It seems most likely that bond fission occurs in all four positions.

The importance of these results for biochemists lies partly in the unexpected complexity of the isotope data and also the fact that they provide a firm basis for the use of ^{18}O tracer in biosynthetic studies of ADP and ATP. The demonstration that isotope exchange is not a concomitant reaction with hydrolysis shows that the common practice of determining the total incorporation of ^{18}O tracer in ATP by complete hydrolysis in acid solution to AMP and inorganic phosphate is justified (Boyer & Bryan, 1966). Any ambiguity about the position of the tracer can, however, only be resolved by using the results presented above.

Acknowledgments

We thank Dr. M. Wilkinson for writing the computer program used on the analysis of the kinetic data.

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Amino Acid Sequence of the Variable Regions of Light Chains from Two Idiotypically Cross-Reactive Human IgM Anti-γ-globulins of the Wa Group†

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ABSTRACT: The amino acid sequences of the variable regions of the light chains derived from two idiotypically related human monoclonal rheumatoid factors are reported. The sequences were obtained through automated Edman degradations of the intact light chains, peptides generated from tryptic digests of citraconylated light chains, and peptides obtained from chymotryptic digestions of light chains. Comparison of the sequences suggests that the idiotypic determinant(s) may reside in the framework portions of the two chains or their J segments.

The immune system is designed to recognize and respond to patterns displayed on molecules encountered by the individual. The generation of the set of recognition units and the subsequent regulation of responsiveness are crucial problems in immunology.

Patterns unique to the individual units themselves ("idiotypes") are ideal candidates as the sites used for both recognition (Capra & Kehoe, 1975) and regulation (Jerne, 1974). Whether these sites are structurally identical is open for debate. In order to approach this question, we have attempted in several systems to generate a structural correlate of the serologically defined idiotype.

Structural correlates of idiotypy have been attempted by using both induced antibodies and pathogenetic immunoglobulins. Structural examination of idiotype-bearing induced

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antibodies is typified in the work involving anti-*p*-azobenzenearsonate antibodies in A/J mice. By use of a particular regimen, mice provoked with the appropriate immunogen respond with a family of antibodies 20–70% of which bear a serologically defined "cross-reactive idiotype". The structural basis for this serological phenomenon was elucidated in a series of experiments (Capra et al., 1975, 1977; Capra & Nisonoff, 1979) in which the variable regions of the heavy and light chains of the antibodies isolated from a partially purified fraction of the idiotype-bearing molecules were sequenced. A homogeneous heavy-chain sequence was observed, including the hypervariable regions. A minimum of three different light chains were observed, but they all shared, within the limits of detection, identical hypervariable regions.

Two groups of monoclonally derived human immunoglobulins were examined to probe the nature of the idiotype: the IgM cold agglutinins and the IgM–IgG "mixed cryoglobulins". Antigenic analysis of the family of cold agglutinins (Williams et al., 1968) showed that there was a correlation between idiotype and specificity. Thus, cold agglutinins directed toward a particular erythrocyte surface determinant shared idiotypic features which were lacking in agglutinins of different specificities.

A similar analysis of the monoclonal rheumatoid factors (Franklin & Frangione, 1971; Kunkel et al., 1973, 1974) allowed the categorization of these molecules into three idiotypically distinct groups: the Wa system, the Po system, and a small collection of unclassified proteins. A correlation with specificity was not possible, but primary structural analysis was undertaken on these molecules to provide another basis for comparison. These studies culminated in the complete sequence of the variable regions of the heavy (Capra & Kehoe, 1974) and light (Klapper & Capra, 1976) chains of two members of the Po system, Lay and Pom. A comparison of these sequences indicates that there are multiple differences in the "framework" portions of both the heavy and light chains. This is especially marked in the light chains, which are members of different variable region subgroups (Lay is V_κI, Pom is V_κIII). The striking observation is that of the six complementarity-determining regions (three in the light chain, three in the heavy chain), four are identical. That two antibodies isolated from unrelated individuals contain sets of identical CDRs[1] is, at the very least, a provocative observation.

The inhibition of idiotype/anti-idiotype interactions with appropriate haptens [e.g., Sher & Cohn (1972) and Brient & Nisonoff (1970)] lent support to the proposition that the idiotype, the combining site, and the hypervariable regions were but three aspects of the same entity (Capra & Kehoe, 1975).

We undertook a similar analysis of two proteins (Sie and Wol) from the Wa idiotypic set of rheumatoid factors in order to test this hypothesis concerning the structural basis for serologically defined idiotypes. These proteins bear cross-reacting idiotypic determinants which are clearly distinct from those shared by Lay and Pom. In this paper, and the following one (Andrews & Capra, 1981), we report the complete amino acid sequence of the variable regions of the light and heavy chains of these two rheumatoid factors.

Experimental Procedures

Materials

Plasma was collected from patients Wol and Sie by standard methods. Plasma from patient Sie was a kind gift of Dr. Marvin Stone.

Methods

Preparation of Proteins. Both cryoglobulins were precipitated at ice-bath temperature, washed 5 times with ice-cold phosphate-buffered saline, dissolved in the same buffer at 37 °C, and filtered through a 2.5 × 100 cm column of Ultrogel AcA34, previously equilibrated in Tris-buffered saline (20 mM Tris-HCl/150 mM NaCl/2 mM EDTA/0.2% NaN_3, pH 8.2).

Reduction and Alkylation. Purified proteins or peptides were completely reduced after dissolution in a buffer consisting of 0.5 M Tris-HCl/7 M guanidine hydrochloride/2 mM EDTA, pH 8.2. The solutions (10–20 mg/mL) were made 30 mM in dithiothreitol and heated at 37 °C for 2 h. The solutions were then treated with either iodo[^{3}H]acetic acid or iodo[^{14}C]acetamide for 5 min, after which time they were made 70 mM in iodoacetamide and allowed to stand at ambient temperature for 15 min. They were then either dialyzed or applied directly to a column.

Amino Acid Analysis. Proteins or peptides in acid-washed test tubes were dissolved in 100 μL of 6 N HCl to which 5 μL of an aqueous solution of 0.1 M phenol had been added. The tubes were sealed in vacuo and heated at 110 °C for 18–20 h. After lyophilization, the hydrolyzed mixtures were examined on a Durrum D-500 instrument.

Citraconylation. Proteins or peptides were dissolved in buffer (5 M guanidine hydrochloride/0.1 M Tris-HCl/2 mM EDTA, pH 9.0) at a concentration of 5–10 mg/mL. The resulting solutions were treated with three 5-μL aliquots of citraconic anhydride (Pierce Chemicals) at 5-min intervals. After the final addition, solutions were filtered through a 2 × 20 cm column of Sephadex G-25F equilibrated in 0.5 M NH_3, and the excluded peak was collected and lyophilized. Unblocking of the lysine residues was achieved by heating the proteins or peptides at 37 °C for 4 h in 40% HCOOH.

Tryptic Digestion. Solutions of proteins or peptides in 1% NH_4HCO_3 were treated with an amount of trypsin corresponding to a 1:100 ratio by weight. The trypsin (Trypsin-TPCK, Worthington Biochemical Corp. was derived from a solution made to a concentration of 10 mg/mL in 1 mM HCl. Solutions were heated at 37 °C for 2 h, followed by the addition of another aliquot of enzyme and another 2 h of reaction. The resulting solutions were then either lyophilized or applied to a column.

High-Voltage Paper Electrophoresis. Electrophoresis was carried out on Whatman No. 1 (analytical) and Whatman No. 3 (preparative) paper in pyridine acetate buffer at pH 3.5 or pH 6.5 under Varsol (Du Pont) in a Savant apparatus (Crumpton & Wilkinson, 1965). Peptides were detected with a 1% ninhydrin–cadmium acetate stain and eluted from No. 3 paper with 0.5 M NH_3.

Automated Amino Acid Sequencing. A Beckman 890C amino acid sequencer, modified by the addition of a cold trap (McCumber et al., 1980), was used in these studies, and its operation with a dimethylallylamine (DMAA) or a Quadrol program using Polybrene (Aldrich Chemical Co., Milwaukee, WI) has been described (Klapper et al., 1978). The resulting phenylthiocarbamoyl amino acid derivatives were converted to their more stable phenylthiohydantoins via reaction with 1 N HCl during 10 min at 80 °C, and the analysis of these compounds was addressed by using gas chromatography (Pisano et al., 1972), high-pressure liquid chromatography (Klapper et al., 1978), and amino acid analysis after hydrolysis with hydriodic acid (Smithies et al., 1971). For the vast majority of these studies the repetitive yields exceeded 95%.

[1] Abbreviations used: CDR, complementarity-determining region; EDTA, ethylenediaminetetraacetic acid; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; Tris, tris(hydroxymethyl)aminomethane.

Table I: Wol κ-Chain Peptides

	CTD-C2 (47-55)		CTD-C4 (56-62)		CTD-C1 (63-78)		CTB-B3 (79-97)		CTD-C5 (98-109)		Chy-D (93-99)	
amino acid	AAA[a]	Seq[b]	AAA	Seq	AAA	Seq	AAA	Seq	AAA	Seq	AAA	Seq
S-CM-Cys[c]							0.8	1				
Asp			1.2	1	1.3	1	1.0	1				
Thr			0.9	1	2.7	3			1.8	2	0.7	1
Ser	1.8	2			4.0	4	1.0	1			0.8	1
Glu							4.4	4	1.9	2		
Pro			0.9	1			1.1	1				
Gly	1.2	1	1.3	1	3.4	3	2.1	2	2.3	2	2.0	2
Ala	1.2	1	1.2	1			1.3	1				
Val							1.1	1	1.1	1		
Met												
Ile	0.7	1	0.8	1	1.0	1			1.0	1		
Leu	1.7	2			1.3	1	2.2	2			1.0	1
Tyr	1.1	1					2.8	3				
Phe					1.7	2	0.9	1	1.0	1	1.4	1
His												
Lys									2.0	2		
Arg	1.1	1	0.8	1	1.0	1	1.0	1	1.4	1	1.1	1
Trp	ND[d]											

[a] AAA = amino acid analysis. [b] Seq = Determined from sequence analysis. [c] *S*-(Carboxymethyl)cysteine. [d] ND = not determined.

For very short peptides, it was often lower.

Chymotryptic Digestion. Solutions of proteins or peptides in 1% NH_4HCO_3 were treated with an amount of chymotrypsin corresponding to a 1:50 ratio by weight. The chymotrypsin (Worthington Biochemical Corp.) was derived from a solution made to a concentration of 10 mg/mL in 1 mM HCl. Solutions were heated at 37 °C for 2 h and then either lyophilized or applied to a column.

Idiotypic Analysis. Protein Sie was originally typed as a member of the Wa group [see Table V, Kunkel et al. (1973)], listed as protein Si, and further studied by Kunkel et al. (1974), listed as protein Sie, where it was classified as a Wa positive, V_H blocked, V_κIIIb protein. Protein Wol was classified as a member of the Wa group in our laboratory by utilizing these same antisera. More recently, both proteins were retyped by Dr. Vincent Agnello, Tufts–New England Medical Center, Boston, MA, and were found to be highly cross-reactive and members of the previously described Wa cross-idiotypic group. In his study, different antisera were used to classify these and other proteins, but the prototypic Wa and Po IgM cryoglobulins were used as reference proteins. The methodology used has been described (Agnello et al., 1980).

Results

Wol Light Chains. A total of 2 mg of Wol light chain was subjected to a sequencer degradation, and unequivocal identifications could be made for 49 cycles of degradation. A tryptic digest of 10 mg of citraconylated Wol light chain was filtered through a column of Ultraogel AcA54 equilibrated in 1% NH_4HCO_3, giving the elution profile in Figure 1. These peptides were labeled CT-A–D.

CT-A and CT-C contained negligible amounts of material and were not investigated. Commitment of a small amount of CT-B to automated sequencing revealed that it contained only constant region peptides by comparison with known κ-chain sequences (Kabat et al., 1979). CT-D was lyophilized, redissolved in 1% NH_4HCO_3, and filtered through a column of Sephadex G-50SF equilibrated in the same solvent, giving the elution profile in Figure 2. These peptides were labeled CTD-A–D.

CTD-A was not investigated, once again because of negligible amounts. Automated sequencing of CTD-B revealed that it was a mixture of three peptides: 1–18, 56–78, and 79–97 (CTB-B1, CTB-B2, and CTB-B3, respectively). These

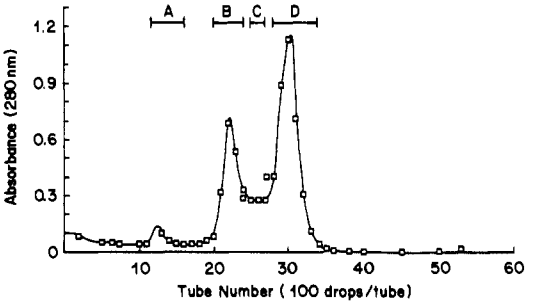

FIGURE 1: Gel filtration of the tryptic digest of citraconylated Wol light chain through Ultrogel AcA54 in 1% ammonium bicarbonate. Column dimensions were 0.8 × 100 cm, and column effluent was monitored by optical density at 280 nm. Fractions were pooled as indicated.

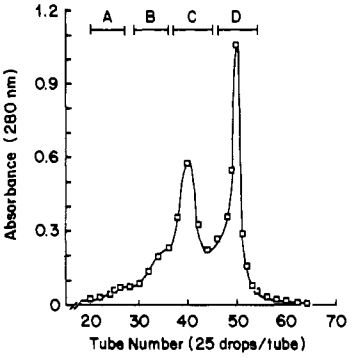

FIGURE 2: Gel filtration of pool D (Figure 1) through Sephadex G-50SF in 1% ammonium bicarbonate. Column dimensions were 0.8 × 100 cm, and column effluent was monitored by optical density at 280 nm. Fractions were pooled as indicated.

peptides were separated by high-voltage paper electrophoresis and separately sequenced. Unequivocal indentifications could be made at each step, and the peptide sequences were easily located in the variable region, both by homology with other κ-chain sequences and with respect to other peptides subsequently sequenced from this digest. CTD-C was subjected to high-voltage paper electrophoresis, from which five peptides could be isolated. These were labeled CTD-C1–C5. CTD-C2 (47–55), CTD-C4 (56–62), CTD-C1 (63–78), and CTD-C5 (98–109) provided data crucial in establishing the sequence of the Wol V_L region (Table I).

In order to confirm the putative sequence in the third

WOLIN LIGHT CHAIN VARIABLE REGION

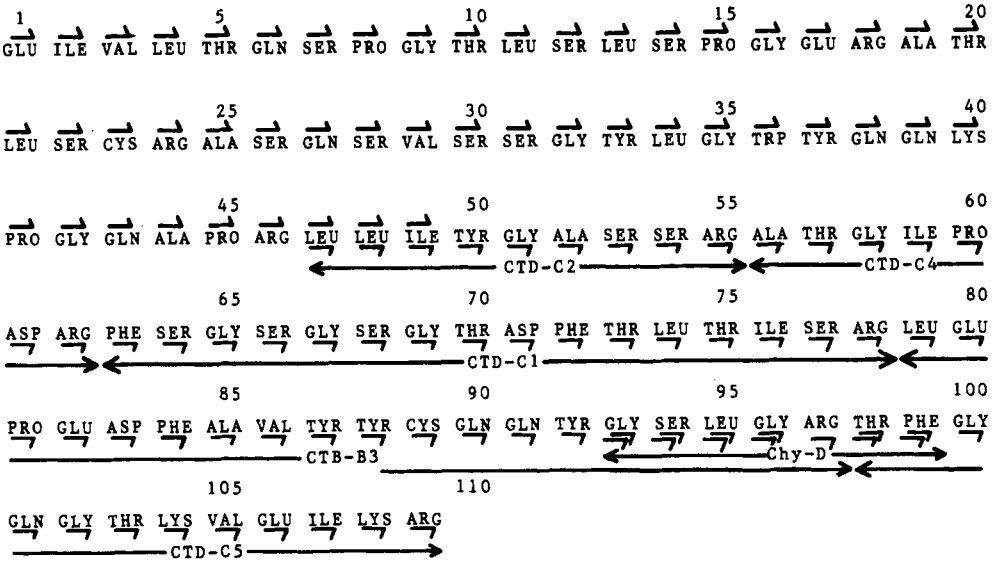

FIGURE 3: Amino acid sequence of the variable region of Wol light chain. Arrows above the sequence represent assignments made on a sequencer run of intact light chain. Arrows below the sequence are assignments made on individual sequencer runs on the isolated peptides. The prefix CT signifies a peptide derived from a tryptic digest of citraconylated light chain. The prefix Chy signifies a chymotryptic peptide.

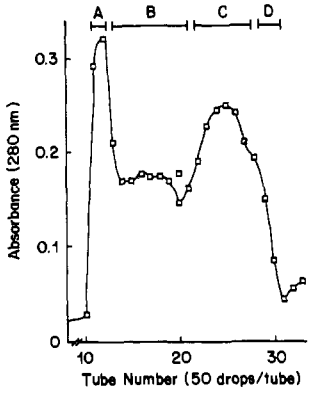

FIGURE 4: Gel filtration of the tryptic digest of citraconylated Sie light chain through Sephadex G-50SF in 1% ammonium bicarbonate. Column dimensions were 0.8 × 100 cm, and column effluent was monitored by optical density at 280 nm. Fractions were pooled as indicated.

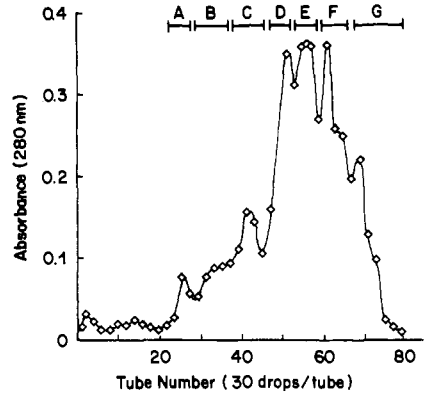

FIGURE 5: Gel filtration of the tryptic digest of incompletely citraconylated Sie light chain through Sephadex G-50SF in 1% ammonium bicarbonate. Column dimensions were 0.8 × 100 cm, and column effluent was monitored by optical density at 280 nm. Fractions were pooled as indicated.

complementarity-determining region of this light chain obtained at the carboxyl terminus of peptide CTB-B3, we sought peptides from a hydrolysis of the light chain catalyzed by chymotrypsin. Filtration of a chymotryptic digest of 5 mg of light chain through a column of Sephadex G-50 afforded six pools, the sequence of one of which (Chy-D) gave the confirmatory and overlap sequence (Table I). The peptides from each of these gel filtrations that were used in deducing the sequence of the variable region of Wol light chain are illustrated in Table I, and the complete sequence of the variable region of the Wol light chain is illustrated in Figure 3.

Sie Light Chain. A total of 2 mg of Sie light chain was subjected to a sequencer degradation, and unequivocal identifications could be made for 30 cycles of degradation. A tryptic digest of 20 mg of citraconylated light chain was filtered through a column of Sephadex G-50SF, equilibrated in 1% NH_4HCO_3, and the elution profile in Figure 4 was obtained. These peptides were labeled CT1-A–D.

CT1-A contained residual uncleaved light chain and constant region peptides. The rest of these pools could be purified by high-voltage paper electrophoresis. They were designated with their original label, followed by a number indicative of their relative electrophoretic mobilities, e.g., CT1-B5.

Another tryptic digest of 10 mg of citraconylated Sie light chain was filtered through a column of Sephadex G-50SF in 1% NH_4HCO_3. These peptides were labeled CT2-A–G. The elution profile (Figure 5) gave the impression that the blockade of lysine residues had been incomplete. This impression was due to the elution of most of the protein-containing material near the total volume of the column, signifying that most of the fragments were quite small. This impression was borne out by the discovery of several peptides involving cleavage at lysine in this digest. The peptides from pools CT2-D–G were purified via high-voltage paper electrophoresis and labeled, once again, with numbers designating their relative electrophoretic mobilities, e.g., CT2-D2.

The following peptides (Table II) provided the data necessary for the determination of the complete sequence of the Sie V_L region: CT1-B3 (25–46), CT1-C3A (47–55), CT1-C3B (56–62), CT2-E1 (63–78), CT2-D1 (79–104), and CT1-B5 (105–109). The complete sequence and peptide alignments are depicted in Figure 6.

Discussion

The sequence data are presented in a comparative manner for the light chains in Figure 7. The light chains of Sie and

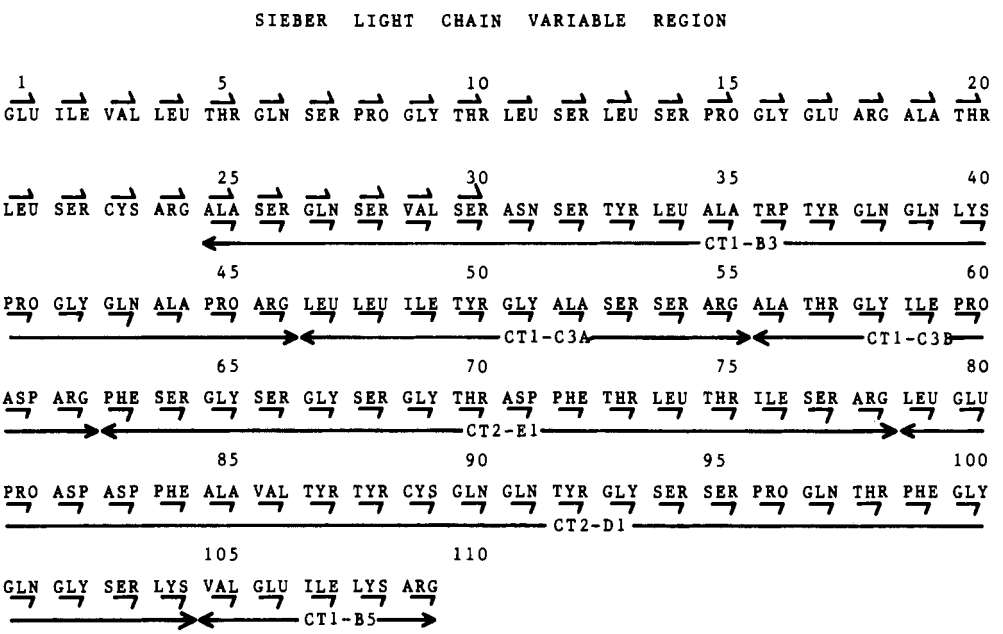

FIGURE 6: Amino acid sequence of the variable region of Sie light chain. Arrows above the sequence represent assignments made on a sequencer run of intact light chain. Arrows below the sequence are assignments made on individual sequencer runs on the isolated peptides. The prefix CT signifies a peptide derived from tryptic digest of citraconylated light chain.

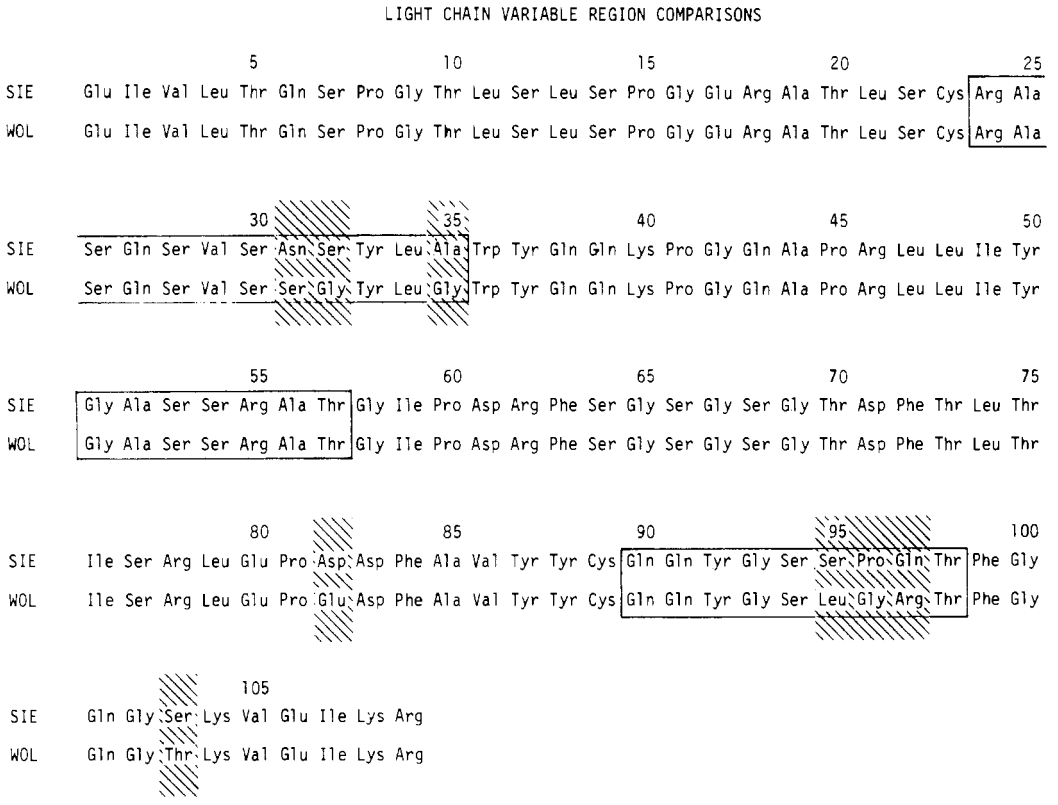

FIGURE 7: Amino acid sequence of the variable regions of the light chains of Sie and Wol, two IgM anti-γ-globulins with shared idiotypy. The differences in the sequences are shaded, and the complementarity-determining regions are boxed.

Wol are remarkably similar. Out of 81 framework residues, there are but two differences between the chains (positions 82 and 103). Within the complementarity-determining regions, Sie and Wol differ at 6 of 28 positions (31, 32, 35, 95, 96, and 97). By focusing on each individual complementarity-determining region, it can be seen that there are three differences in CDR I, none in CDR II, and three in CDR III. Among the V_κIII proteins listed in a computer compilation of immunoglobulin sequences (Kabat et al., 1979), the average number of differences in CDR I compared with the prototype, Ti (derived from a nonrheumatoid factor), is three. In CDR II, the average number of differences is one, and in CDR III, the average is four. The average difference in complementarity-determining regions among this light-chain subgroup is eight, while Wol and Sie differ at six positions. Thus, the CDRs of Wol and Sie light chains are slightly more similar (by this measure) than two randomly chosen human V_κIII proteins.

As noted above, the framework sections of these two light chains are remarkably similar. The differences between the two chains are structurally conservative as well (position 82, Asp/Glu; position 103, Ser/Thr). In fact, all the light chains of the Wa group of rheumatoid factors are members of a very

Table II: Sie κ-Chain Citraconylated Tryptic Peptides

	CT1-B3 (25–46)		CT1-C3A (47–55)		CT1-C3B (56–62)		CT2-E1 (63–78)		CT2-D1 (79–104)		CT1-B5 (105–109)	
amino acid	AAA[a]	Seq[b]	AAA	Seq	AAA	Seq	AAA	Seq	AAA	Seq	AAA	Seq
S-CM-Cys[c]									ND	1		
Asp	0.9	1			1.0	1	1.6	1	2.0	2		
Thr					0.8	1	1.8	3	1.1	1		
Ser	3.6	4	1.6	2			2.0	4	2.3	3		
Glu	4.2	4							4.5	5	1.3	1
Pro	1.9	2			1.0	1			2.0	2		
Gly	1.4	1	1.4	1	1.3	1	3.0	3	2.8	3		
Ala	3.2	3	1.1	1	1.1	1			1.2	1		
Val	1.0	1							1.3	1	0.9	1
Met												
Ile			1.0	1	1.8	1	0.8	1			0.8	1
Leu	1.0	1	2.4	2			0.9	1	1.3	1		
Tyr	0.9	2	1.0	1					1.9	3		
Phe							1.8	2	1.6	2		
His												
Lys	1.0	1							0.8	1	0.8	1
Arg	0.9	1	ND	1	0.7	1	0.8	1			0.8	1
Trp	ND[d]	1										

[a] AAA = amino acid analysis. [b] Seq = determined from sequence analysis. [c] *S*-(Carboxymethyl)cysteine. [d] ND = not determined.

homogeneous subdivision of the V_κIII subgroup called V_κIIIb [defined by McLaughlin & Solomon (1972)]. This subsubgroup is present in 11% of normal immunoglobulins and only 8% of nonrheumatoid factor IgMs (Kunkel et al., 1974). The fact that 100% of the proteins of the Wa group carry these light chains, and none of the Po group has them, makes it difficult to dismiss this set of determinants as the predominant idiotypic contributor.

Adsorptions of anti-idiotypic antisera with concentrated pooled normal human immunoglobulin might not be sufficient to remove this specificity, especially if the determinant were formed by the combination of V_HI heavy chains and V_κIIIb light chains. An attempt was made to refute this argument by Kunkel et al. (1974), who tried to inhibit the idiotype/anti-idiotype reacton with other V_κIIIb-bearing, nonrheumatoid factor immunoglobulins. There was no inhibition with any of the proteins used, including "...one IgM with IIIb-type light chains and blocked heavy chains". Given the large differences among V_HI heavy chains [see Discussion in the following paper in this issue (Andrews & Capra, 1981)] and the fact that subgroups other than V_HI may have blocked heavy chains, the possibility still exists that the idiotype in this system is formed by the framework residues of the heavy and light chains, acting in concert.

The portions of these two light chains corresponding to the J segment constitute another potential site for idiotypic determinants. The movement of the DNA encoding the V segment (residues 1–96) to a position contiguous with one of several J segments (residues 97–109) has been extensively analyzed in the mouse λ chain (Seidman et al., 1978; Bernard et al., 1978; Sakano et al., 1979; Max et al., 1979). It is clear that the J segment and its contiguous DNA sequences contain information critical for at least two processes: the proper joining of V and J and the appropriate editing of the L/V/J-C transcript. It also appears that latitude in the site for recombination during the V/J joining step may be important in generating diversity in this portion of the light chain.

In human light chains, the J segment has been inferred from sequence data and defined by analogy with the murine system as extending from residues 97–109, inclusive. This portion of these light chains is illustrated in Figures 8 and 9, along with the same portion from two members of the other idiotypic set, Lay and Pom.

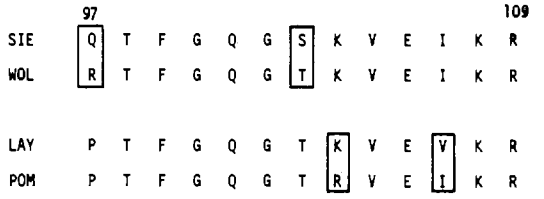

FIGURE 8: J segments of the light chains of four IgM anti-γ-globulins. Sie and Wol share idiotypic specificities which are different from those shared by Lay and Pom. The differences *within* each set are boxed.

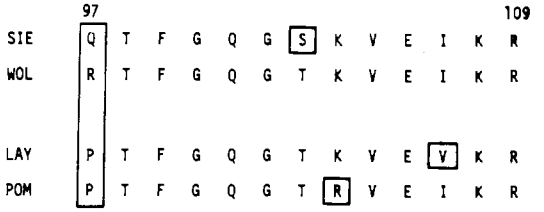

FIGURE 9: J segments of the light chains of four IgM anti-γ-globulins. Sie and Wol share idiotypic specificites which are different from those shared by Lay and Pom. The differences *between* the two sets are boxed.

The J segments of Sie and Wol are quite similar, differing at 2 of the 13 positions, 97 and 103. These substitutions are structurally conservative (97, Gln/Arg; 103, Ser/Thr). Lay and Pom also differ at two positions, 104 and 107, and these substitutions are likewise conservative (104, Lys/Arg; 107, Val/Ile).

Between the two idiotypic sets, the differences are as follows: Sie/Lay, 97, 103, and 107; Sie/Pom, 97, 103, and 104; Wol/Lay, 97 and 107; Wol/Pom, 97 and 104. The substitutions are all conservative, except for the first, at position 97. In Lay and Pom this residue is a proline, while in Sie it is a glutamine and in Wol an arginine. Glutamine and arginine, while differing in charge, are quite similar in size. Proline, however, has much different characteristics than either of these, and, additionally, it induces turns in polypeptide chains.

Thus, the J segment of the light chain is quite similar between Sie and Wol, as well as between Lay and Pom. Second, the differences noted between the members of these two idiotypic sets could provide the structural deviation perceived as an idiotypic determinant in this system. It may be more than coincidence that a potential idiotypic determinant is

generated at the V/J junction, a point of high variability.

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Amino Acid Sequence of the Variable Regions of Heavy Chains from Two Idiotypically Cross-Reactive Human IgM Anti-γ-globulins of the Wa Group†

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ABSTRACT: The amino acid sequences of the variable regions of the heavy chains derived from two idiotypically related human monoclonal rheumatoid factors are reported. The sequences were obtained through automated Edman degradations of the intact, pyroglutamate aminopeptidase cleaved heavy chains and peptides produced from digestion of the pentameric IgM with CNBr. The peptides generated from the CNBr reaction were further digested by trypsin and the *Staphylococcus aureus* V8 protease. Comparisons of these sequences and those of the light chains from these molecules [Andrews, D. W., & Capra, J. D. (1981) *Biochemistry* (preceding paper in this issue)] suggest that the idiotypic determinant(s) in this system may reside in the framework portions of these molecules or in their J segments.

In order to further our objective of defining a structural correlate of a serologically defined idiotype, we have sequenced the variable regions of the heavy chains of two idiotypically related rheumatoid factors. Previous structural analysis of two proteins from the Po cross-idiotypic group (Capra & Kehoe, 1974; Klapper & Capra, 1976) provided strong evidence that the CDRs of the light and heavy chains of these molecules generated the predominant idiotypic determinant in this system. Our initial studies on the light chains of two proteins from the Wa cross-idiotypic group (Andrews & Capra, 1981) did not confirm this view.

Since the idiotype in this system is dependent on both the heavy and light chains (Kunkel et al., 1973, 1974), the complete variable region sequences of both the heavy and light

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