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Amino Acid Sequence of the *N*-Terminal 139 Residues of Light Chain Derived from a Homogeneous Rabbit Antibody

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The amino acid sequence of the *N*-terminal 139 residues of the L (light) chain derived from a homogeneous rabbit antibody (designated BS-1) to type III pneumococci was determined. A combination of methods involving tryptic cleavage restricted to the 2 arginine residues of the molecule and mild acid hydrolysis of a labile peptide bond between the V (variable) and C (constant) regions of the L chain (Fraser *et al.*, 1972) allowed the isolation of two large peptides comprising the entire V region (residues 1–109); these peptides were suitable for automated Edman degradation. The complete sequence analysis of the V region was carried out with only 4μmol of L chain. This material was homogeneous, although minor variant sequences, if present at the 10% value, would not have been detected. The L chain contains 3 intrachain disulphide bridges, whose pairing was established by diagonal electrophoresis: there is one V-region bridge between positions 23 and 88 and one C-region bridge between positions 134 and 194; the third one connects V and C domains between positions 80 and 171. When compared with the basic sequence of human κ chains, rabbit L chain BS-1 appears to be more similar to the V_{KI} prototype sequence than to V_{KII} or V_{KIII} sequences, where V_{KI}, V_{KII} and V_{KIII} represent subgroups I, II and III respectively of V regions of κ light chains. The V regions of rabbit heavy and light chains are homologous to each other. The presence of two clusters of 3 glycine residues in positions 94–96 and 99–101 respectively is remarkable. Residues 94–96 may be related to antibody complementarity whereas residues 99–101 function probably as a pivot permitting the combining region of the L chain to make optimal contact with the antigenic determinant (Wu & Kabat, 1970).

Amino acid sequence studies of a large number of myeloma proteins have shown that both the heavy (H)* and light (L) chains of immunoglobulins comprise a variable *N*-terminal half (V region) of 106–110 residues and an invariant *C*-terminal half (C region) (for review, see Edelman & Gall, 1969).

Homogeneous antibodies stimulated by bacterial polysaccharide antigens are suitable material for study of the primary structure of both H (Fleischman, 1971, 1973) and L chains (Hood *et al.*, 1970; Jaton *et al.*, 1971; Kindt *et al.*, 1972; Braun & Jaton, 1973). On the basis of the available data, sequence variability between anti-polysaccharide antibodies was observed within sections 31–34 and 47–62 of the H chains (Jaton & Braun, 1972). In contrast, section 80–94 of a_1, a_2 and a_3 homogeneous H chains showed little variation (Jaton *et al.*, 1973).

* Abbreviations IgG, immunoglobulin G; H and L chains, heavy and light chains of IgG; V and C regions, variable and constant regions of IgG respectively; V_{KI}, V_{KII} and V_{KIII}, subgroups I, II and III respectively of V regions of κ light chains; V_L and C_L, V and C regions of L chains; Fab′, pepsin fragment which consists of a complete L chain and of the *N*-terminal half of the H chain. CmCys, $\square$Glu (in amino acid sequences and Tables), *S*-carboxymethylcysteine and pyrrolidonecarboxylic acid respectively.

No complete sequences of V regions of rabbit L chains have yet been determined; however, partial sequences from several pneumococcal antibody L chains, as well as from non-immune pooled chains, have been reported (Strosberg *et al.*, 1972). Similarly, half-cystine-containing peptides representing the intrachain disulphide bonds of an L chain derived from a restricted anti-azobenzoate antibody have been described (Appella *et al.*, 1971). The knowledge of the primary and tertiary structures of rabbit L chain is of great interest not only for the structure–function relationship but also for the location of the group b allotypic markers which, in contrast with their counterpart H chains, seem to be present in the C region (Appella *et al.*, 1969; Frangione, 1969; Kindt *et al.*, 1972), even though it was suggested that the *N*-terminal sequence also correlates with each L-chain allotype (Hood *et al.*, 1971).

In the present investigation, I report the amino acid sequence of the V region and of the beginning of the C domain of the L chain derived from a homogeneous rabbit antibody to type III pneumococci. This antibody, of allotypes a_2, b_4, designated BS-1, is specific for the simple antigenic determinant, cellobiuronic acid, the repeating disaccharide of the capsular pneumococcal polysaccharide type III.

Experimental

Materials

Trypsin treated with L-1-chloro-4-phenyl-3-tosylaminobutan-2-one, carboxypeptidases A and B treated with di-isopropyl fluorophosphate, α-chymotrypsin (three times crystallized) and thermolysin were purchased from Worthington Biochemical Corp., Freehold, N.J., U.S.A. Dithiothreitol (A grade) was from Calbiochem, Los Angeles, Calif., U.S.A., iodo[2-^{14}C]acetic acid (specific radioactivity 30mCi/mmol) was from The Radiochemical Centre, Amersham, Bucks., U.K. and hydriodic acid (65%, v/v), stabilized with 0.03% (w/v) H_3PO_4, was from BDH Chemicals Ltd., Poole, Dorset, U.K. 4-Sulphophenyl isothiocyanate (sodium salt, monohydrate, sequanal grade) was obtained from Pierce Chemical Co., Rockford, Ill., U.S.A. and citraconic anhydride was from Aldrich Chemical Co., Milwaukee, Wis., U.S.A. Sequencer-grade reagents and solvents used in the automated Edman degradation were purchased from Beckman International, Geneva, Switzerland. All other reagents and solvents were of the best grade available.

Methods

Antibody production and purification. Rabbit BS-1 was immunized with type III pneumococcal vaccine by a scheme described by Kimball *et al.* (1971). The antibody which showed mainly a single band in the γ-globulin region was isolated with an immunoadsorbent by the method of Jaton *et al.* (1971). It was repurified by preparative agarose block electrophoresis to eliminate 10–15% of a fast-moving component. Details of the method of purification and preparation of H and L chains have already been described (Jaton & Braun, 1972). The antibody BS-1 carried a_2,b_4 allotypes.

Complete reduction of the light chain. The light chain (100mg) was fully reduced and alkylated with iodo-[2-^{14}C]acetic acid by the technique of O'Donnell *et al.* (1970).

Citraconylation of the reduced and [2-^{14}C]alkylated L chain. This was done essentially by the method of Gibbons & Perham (1970). The freeze-dried L chain (100mg) was dissolved in 7M-guanidine hydrochloride (10ml) and a 25-fold molar excess of citraconic anhydride with respect to lysine residues was added gradually within 45min; the pH of the reaction mixture was maintained between 8.5 and 9.0 by dropwise addition of 5M-NaOH solution. Excess of reagents and salts were removed by dialysis against water and the protein was freeze-dried. The extent of modification of amino groups was determined by the trinitrobenzenesulphonic acid test (Habeeb, 1966).

Dilute acid hydrolysis. Large citraconyl-fragment A (1–2μmol) derived from L chain BS-1 was subjected to acid hydrolysis in either 70% (v/v) formic acid (2ml), or in 7M-guanidine hydrochloride containing 10% (v/v) acetic acid, pH2.5 (2ml), for 120h at 37°C as suggested by Fraser *et al.* (1972).

Unblocking of citraconyl-peptides. The citraconyl-fragment B was incubated in 1–2ml of acetate buffer, pH4.2, for 6h at 37°C, similarly to the procedure of Habeeb & Atassi (1970); the solution was then dialysed for 12h against several changes of water and the content of the bag freeze-dried. No appreciable losses of material occurred during dialysis.

Analytical methods. Preparative high-voltage paper electrophoresis at pH6.5 was done as described by Press *et al.* (1966). Neutral peptides were rerun at pH3.5. Diagonal electrophoresis of peptic digest of L chain BS-1 (15mg) at pH6.5 was carried out by the method of Brown & Hartley (1966): peptides were eluted from the paper with 0.02M-NH_3 after light staining with ninhydrin solution in acetone (0.05%, w/v). Peptides were detected by the ninhydrin–cadmium stain (Dreyer & Bynum, 1967), and those containing tyrosine, tryptophan and arginine by specific stains (Smith, 1960). Radioactive peptides were revealed by radioautography for 16h by using Kodak Royal Blue Medical X-ray film.

Enzymic digestions of 50 to 300nmol of peptides with trypsin and α-chymotrypsin were carried out for 3h at 37°C, by using 40–100μg of enzyme in the presence of 0.4–1.5ml of 1% (w/v) NH_4HCO_3. Peptic digestion was done in 5% (v/v) formic acid for 16h at 37°C and thermolysin digestion was done at an enzyme/protein ratio of 1:500 in 1% (w/v) NH_4HCO_3 for 1h at 25°C. Hydrolyses with carboxypeptidases were conducted as recommended by Ambler (1967).

Amino acid analyses on a Beckman model 121 Amino Acid Analyzer and the determination of sequences of small peptides by the 'dansyl–Edman' procedure (Gray, 1967) were as described by Jaton & Braun (1972). Whenever possible, amide residues were assigned on the basis of electrophoretic mobility at pH6.5 (Offord, 1966).

The determination of the amino acid sequence of sections of L chain and of large peptides was done in the Beckman sequencer model 890B, equipped with an undercut cup and N_2 flush. Protein or peptides (250–600nmol) were dissolved in 0.4ml of 1M-acetic acid and degraded by using the conventional Quadrol programme (Edman & Begg, 1967). Alternatively, large tryptic peptides (50–60 residues) were first manually treated with an excess of 4-sulphophenyl isothiocyanate to prevent losses during automated sequential degradation (Braunitzer *et al.*, 1970). In a typical experiment, the peptide (600 nmol) was dissolved in 400μl of dimethylallylamine buffer and incubated for 1h at 63°C in the presence of 50μl of 3% (w/v) 4-sulphophenyl isothiocyanate (sodium salt) in water. The reaction mixture was then transferred to the cup of the sequencer and dried down; a peptide programme utilizing the volatile buffer

dimethylallylamine was started at the cleavage step with heptafluorobutyric acid. The programme was adapted from the original one designed by Beckman, Spinco Division. The identification of the phenylthiohydantoin-amino acid derivatives was accomplished by: (1) g.l.c. (Pisano & Bronzert, 1969) and (2) amino acid analysis after conversion of the phenylthiohydantoin derivatives into free amino acids by hydrolysis with 65% (w/v) HI for 20h at 125°C (Smithies *et al.*, 1971), as described by Jaton & Braun (1972). The overall repetitive yield in sequencing experiments varied from 90% for fragments A and A_3 to 94% for fragments A_1 and B respectively, and the absolute yield of the phenylthiohydantoin-amino acid derivatives at the first step ranged between 22 and 56%.

Radioactivity measurements were carried out in vials containing 10ml of scintillation liquid (Bray, 1960) in a Nuclear–Chicago liquid-scintillation counter.

Results

Isolation and characterization of peptides from a tryptic digest of [2-^{14}C]carboxymethylated and citraconylated L chain BS-1

L chain BS-1 contains only 2 arginine residues/molecule (Table 1). Advantage was taken of this fact by cleaving the L chain with trypsin after lysine residues had been blocked with citraconic anhydride. The components of the tryptic digest of [2-^{14}C]-carboxymethylated and citraconylated L chain were separated by gel filtration into two fractions, A and B (Fig. 1). A portion of radioactive material under peaks A and B was not dialysable. Fractions A and B were thus dialysed overnight against water to remove excess of salts and freeze-dried. The amino acid composition (Table 1) indicates that fragment A contains about 150 amino acids including 5 half-cystine residues, whereas fragment B has 61 residues among which is 1 half-cystine. Both have arginine at the *C*-terminal position. Three fractions are expected to occur in a tryptic digest of citraconylated L chain containing 2 arginine residues. Two arginine peptides were accounted for; the third fraction is likely to contain the *C*-terminal tripeptide Gly-Asp-Cys in positions 212–214 (Appella *et al.*, 1969), which escaped detection under our experimental conditions. On the basis of 4.3μmol of L chain, peptides A and B were recovered in 80% and 82% yield respectively. Citraconyl groups were removed from fragment B and the resulting deblocked peptide (600nmol) was subjected to automated sequential degradation after prior treatment with 4-sulphophenyl isothiocyanate (see under 'Methods'). The amino acid sequence of the first 37 residues of fragment B (Fig. 2) is identical with the *N*-terminal sequence of L chain BS-1 determined by Braun & Jaton (1973). It is

Table 1. *Amino acid composition of the citraconyl peptides isolated from a tryptic digest of reduced and carboxymethylated L chain BS-1*

Values are residues/molecule of peptide. Values in parentheses are integral values confirmed by sequence analysis. Fragments A_1 and A_3 were released from citraconyl fragment A after treatment in dilute acid at 37°C (see the text). N.D., not determined.

Amino acid	Fragment A	Fragment A_1	Fragment A_3	Fragment B	Carboxymethylated L chain
Lys	6.1	4.0	2.0 (2)	4.2 (4)	10.5
His	1.0	0.9	—	—	1.5
Arg	1.1	0.8	—	1.2 (1)	2.2
CmCys	5.6	3.2	1.8 (2)	1.2 (1)	6.6
Asp	16.6	13.8	3.3 (3)	1.1 (1)	17.9
Thr	23.0	17.5	6.0 (6)	4.8 (5)	27.8
Ser	14.2	7.9	3.9 (4)	9.1 (9)	22.5
Glu	13.8	8.9	4.2 (4)	7.0 (7)	20.0
Pro	6.3	6.2	0.3 (0)	5.2 (5)	11.0
Gly	14.3	3.1	9.8 (11)	4.6 (5)	18.8
Ala	9.9	6.0	3.0 (3)	5.1 (5)	13.8
Val	13.8	11.0	3.2 (3)	5.6 (6)	20.1
Met	—	—	—	0.9 (1)	1.2
Ile	3.6	2.8	1.1 (1)	3.1 (3)	7.2
Leu	6.1	3.6	2.0 (2)	4.2 (4)	10.1
Tyr	6.7	3.8	2.9 (3)	3.1 (3)	10.1
Phe	7.8	3.6	3.8 (4)	— (0)	8.0
Trp	N.D.	N.D.	—*	+ (1)	+ †
Total residues	148.8	97.1	47.3	60.4	209.3

* Measured with Ehrlich's reagent.
† Excluding tryptophan.

concluded that fragment B is the *N*-terminal 61-residue peptide of the L chain. Consequently, the second arginine-containing peptide, A, should follow fragment B and extend between positions 62 and 211.

Citraconyl-fragment A (500nmol) was subjected directly to automated Edman degradation by using the Quadrol programme. A single amino acid derivative was found at each of the *N*-terminal 28 positions (Fig. 2). An analysis of yield at each step is shown in Fig. 3. The absolute yield of phenylthiohydantoin-phenylalanine at the first step was 56%. The drastic fall in yield of phenylthiohydantoin-amino acids of 90% over 27 residues prevented further degradation of peptide A. The completion of the determination of the sequence of peptide A_3 by conventional methods is described below. The *N*-terminal sequence of fragment A is Phe-Lys-Gly-Ser-Gly-Ser-Gly-Thr, which is characteristic of most κ light chains and occurs after arginine in position 61 (Putnam *et al.*, 1967). Of special interest is the identification of 2 half-cystine residues in positions 19 and 27 (Fig. 3), i.e. in corresponding positions 80 and 88 respectively of the L chain (Fig. 6). The significance of the cysteine residue at position 80 is discussed below.

Partial sequence of fragment A

Fragment A was further subjected to dilute acid hydrolysis, as it has been shown that a specific

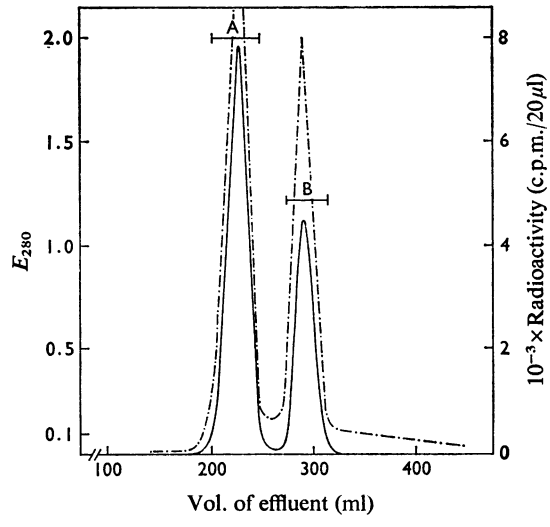

Fig. 1. *Elution profile of a tryptic digest of* [2-^{14}C]*carboxymethylated and citraconylated L chain BS-1*

The digest (100mg) was loaded on a column (2cm × 200cm) of Sephadex G-50 (fine grade) and developed in 6M-guanidine hydrochloride containing 0.1M-Tris–HCl buffer, pH8.0. See the text for details. ——, E_{280}; –·–·–, radioactivity (c.p.m./20μl of eluate).

Residue position	1								10										20
L-chain BS-1*	Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Ala	Ser	Val	Ser	Glu	Pro	Val	Gly	Gly	Thr	Val	Thr
Fragment B																			
													70						80
Fragment A	Phe	Lys	Gly	Ser	Gly	Ser	Gly	Thr	Glu	Phe	Thr	Leu	Thr	Ile	Ser	Asp	Leu	Glu	Cys†	Ala
Fragment A_3																			
	110										120								
Fragment A_1	Pro	Val	Ala	Pro	Thr	Val	Leu	Ile	Phe	Pro	Pro	Ala	Ala	Asp	Gln	Val	Ala	Thr	Gly	Thr

Residue position										30					35				
L-chain BS-1*	Ile	Lys	Cys†	Gln	Ala	Ser	Gln	Ser	Ile										
Fragment B																			
	Tyr	Ser	Gly	Leu	Ala	Trp	Tyr	Glx											
Fragment A	Asx	Ala	Ala	Thr	Tyr	Phe	Cys†	Glx											
Fragment A_3																			
	130																		
Fragment A_1	Val	Thr	Ile	Val	Cys†	Val	Ala	Asn	Lys	Tyr									

Fig. 2. *Automated sequential degradations of L chain BS-1 and fragments A, A_1, A_3 and B derived thereof*

L chain BS-1 (250nmol), fragments B (600nmol), A (500nmol), A_3 (300nmol) and A_1 (450nmol) respectively were used for sequence determination. A conventional Quadrol programme was used for L chain BS-1, fragments A and A_1, whereas a volatile buffer system and sulphophenyl isothiocyanate were employed for peptides B and A_3 (see under Methods for details). The solid line indicates identical sequence with that shown immediately above. The numbering of the residues in the fragments indicates their position in the complete sequence.

* From Braun & Jaton (1973).

† Determined by counting a portion of the phenylthiohydantoin-*S*-carboxymethylcysteine derivative for radioactivity (see also Fig. 3).

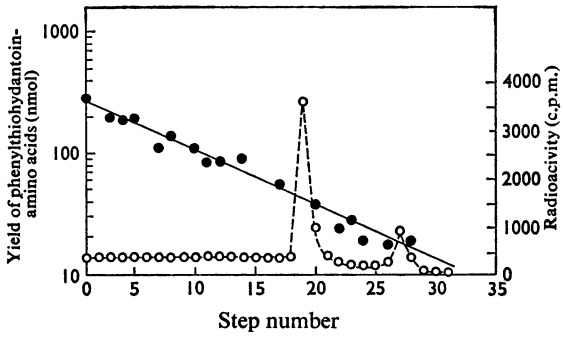

Fig. 3. *Quantitative yields of phenylthiohydantoin-amino acids obtained at each step of the automated Edman degradation of fragment A*

Yields were computed by comparing peak heights of samples with those of relevant standard derivatives by g.l.c. (●) and c.p.m. of a 5% sample of the phenylthiohydantoin-amino acid at each step (○). Radioactivity at steps 19 and 27 indicates a *S*-carboxymethylcysteine residue. The absolute yield of phenylthiohydantoin-phenylalanine at the first step was 56%, on the basis of 500nmol of fragment A.

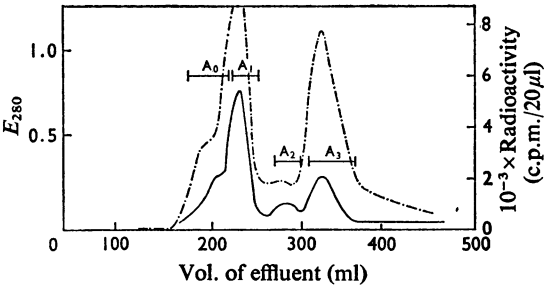

Fig. 4. *Separation of the cleavage products of fragment A subjected to dilute acid hydrolysis*

Fragment A was treated in 7M-guanidine hydrochloride containing 10% acetic acid, pH2.5, for 120h at 37°C. The digest (50mg) was loaded on a column (2cm×200cm) of Sephadex G-50 (fine grade) and developed in 5M-guanidine hydrochloride, pH5.5. See the text for details. ——, E_{280}; -·-·-, radioactivity (c.p.m./20µl of eluate).

cleavage of the labile bond Asp_{109}–Pro_{110} between V and C domains of rabbit L chains can occur (Fraser *et al.*, 1972). The separation of the cleavage products is depicted in Fig. 4. Two major fractions designated A_1 and A_3 were obtained. Fractions A_0 and A_2 probably contain aggregated and/or uncleaved material, present in small amounts, and were not analysed further. Peptides A_1 and A_3 were dialysed overnight against several changes of water and freeze-dried. No significant loss of material was found after dialysis.

Peptide A_3 comprises 48 amino acids among which are 2 half-cystine residues but no arginine (Table 1). On the basis of 3µmol of fragment A, peptide A_3 was recovered in 65% yield. Its *N*-terminal sequence (Fig. 2) is identical with that of fragment A from which it was derived, indicating that fraction A_3 contains the V-region peptide extending from positions 62–109.

The other fragment, A_1, has about 100 amino acids on the basis of 1 arginine residue (Table 1). Its yield was 63%. Arginine was determined to be the *C*-terminal residue by carboxypeptidase B digestion and proline to be the *N*-terminal residue by the dansylation technique. This suggests that the mild acid cleavage of fragment A occurred at the bond between aspartic acid 109 and proline 110. The purity of the large fragment A_1 is indicated by the single sequence found in its first *N*-terminal 30 positions (Fig. 2). This sequence, homologous to human κ chains (Putnam *et al.*, 1967) and identical with other rabbit L chains (Strosberg *et al.*, 1972), proceeds starting with residue 110 at the beginning of the constant region. Fragment A_1 therefore comprises the constant domain (residues 110–211) with the exception of the three *C*-terminal residues of the L chain.

The ordering of the citraconyl fragments and of the peptides derived from them is summarized in Fig. 5. The entire V region comprises two peptides: fragments B and A_3, whereas the C domain is made up of the large fragment A_1 plus the *C*-terminal tripeptide.

Sequence determination of fragment A_3

Fragment A_3 was briefly digested with thermolysin (see under 'Methods'). Five major peptides designated Th_1, Th_2, Th_3, ThN_1 and ThN_2 were isolated by paper electrophoresis in satisfactory yield. Their characterization and sequence are described in Table 2 and Fig. 6.

Peptide ThN_1 is a tetrapeptide with the sequence Phe-Thr-Leu-Thr.

The *N*-terminal sequence of the nonapeptide ThN_2 was Phe-Lys-Gly-Ser; this and its amino acid composition indicate that peptide ThN_2 is the *N*-terminal peptide of fragment A_3.

Peptide Th_1 is a dodecapeptide rich in glycine and valine, which contains 1 lysine residue. After tryptic cleavage two peptides were obtained: the *N*-terminal decapeptide $T\text{-}Th_{1b}$ and the *C*-terminal dipeptide Gly-Asp. The sequence of peptides Th_1 and $T\text{-}Th_{1b}$ is shown in Fig. 6.

Peptide Th_2 has the *N*-terminal sequence Phe-CmCys-Glx-Gly-Ser-Thr-Tyr. The position of *S*-carboxymethylcysteine in this 11-residue peptide was confirmed by the removal of 85% of the radioactivity in the peptide after the second degradation

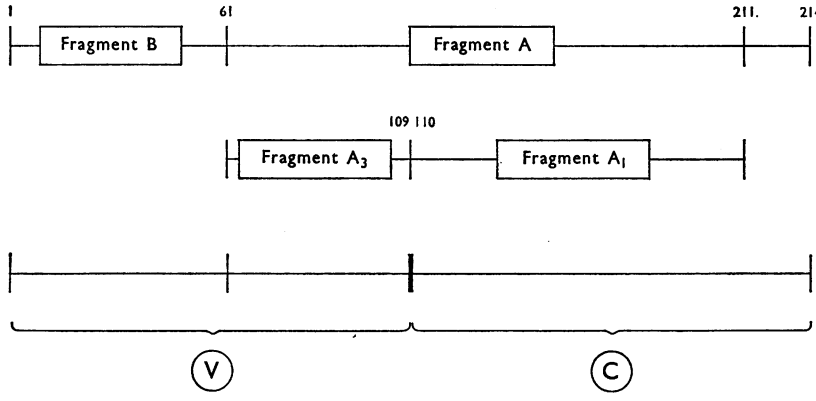

Fig. 5. *Alignment of the citraconylated peptides derived from L chain BS-1*

Fragments A and B were isolated from a tryptic digest of carboxymethylated and citraconylated L chain. Fragments A_1 and A_3 were derived from fragment A after dilute acid hydrolysis (see the text). V and C denote the variable and constant halves of the L chain.

Table 2. *Amino acid composition and properties of constituent peptides of the fragment A_3 of L chain BS-1*

Values are residues/molecule of peptide. Values in parentheses are integral values confirmed by sequence analysis. Thermolysin and chymotryptic peptides were derived from fragment A_3. Peptides Ch-Th_{2a} and Ch-Th_{2b} were derived from a chymotryptic digest of peptide Th_2. Peptides T-Th_{1a} and T-Th_{1b} were derived from peptide Th_1 by trypsin digestion. Mobilities are expressed relative to Asp (= −1.0). Yields are based on 1100nmol of fragment A_3.

	Thermolysin peptides					Chymotryptic peptides		Tryptic peptides	
Amino acid	Th1	Th2	Th3	ThN_1	ThN_2	Ch-Th_{2a}	Ch-Th_{2b}	T-Th_{1a}	T-Th_{1b}
Lys	1.1	—	—	—	1.0	—	—	—	1.0 (1)
Arg	—	—	—	—	—	—	—	—	—
CmCys*	—	0.8 (1)	0.9	—	—	—	0.7 (1)	—	—
Asp	1.0	—	2.1	—	—	—	—	1.0 (1)	—
Thr	1.1	0.8 (1)	0.9	2.2 (2)	1.2	—	0.8 (1)	—	0.9 (1)
Ser	—	1.2 (1)	1.1	—	1.9	—	1.2 (1)	—	—
Glu	1.0	1.1 (1)	1.0	—	1.3	—	1.2 (1)	—	1.0 (1)
Pro	—	—	—	—	—	—	—	—	—
Gly	3.6	4.0 (4)	—	—	2.9	2.9 (3)	0.8 (1)	0.9 (1)	2.8 (3)
Ala	—	—	2.9	—	—	—	—	—	—
Val	2.8	—	—	—	—	—	—	—	2.9 (3)†
Ile	—	—	0.9	—	—	—	—	—	—
Leu	—	—	1.0	0.8 (1)	—	—	—	—	—
Tyr	—	1.8 (2)	1.0	—	—	1.0 (1)	0.9 (1)	—	—
Phe	1.0	0.9 (1)	—	1.0 (1)	0.9	—	1.1 (1)	—	0.9 (1)
Total residues	12	11	12	4	9	4	7	2	10
Mobility at pH 6.5	−0.22	−0.48	−0.72	0	0	0	−0.68	−0.79	0
Yield (%)	29	31	35	29	35	29	25	12	15

* Value not corrected for losses during hydrolysis.

† Value after 96h hydrolysis.

step. Peptide Th_2 was further fragmented with α-chymotrypsin and gave rise to two peptides: Ch-Th_{2a} and Ch-Th_{2b}. The sequence of peptide Ch-Th_{2b} is identical with that of peptide Th_2 in its *N*-terminal section. The tetrapeptide Ch-Th_{2a} should therefore be the *C*-terminal part of the peptide Th_2; it has the sequence Gly-Gly-Gly-Tyr.

The *N*-terminal sequence Ile-Ser-Asp-Leu-Glu-Cys of the dodecapeptide Th_3 indicates that it is the cysteine-80-containing peptide of fragment A_3.

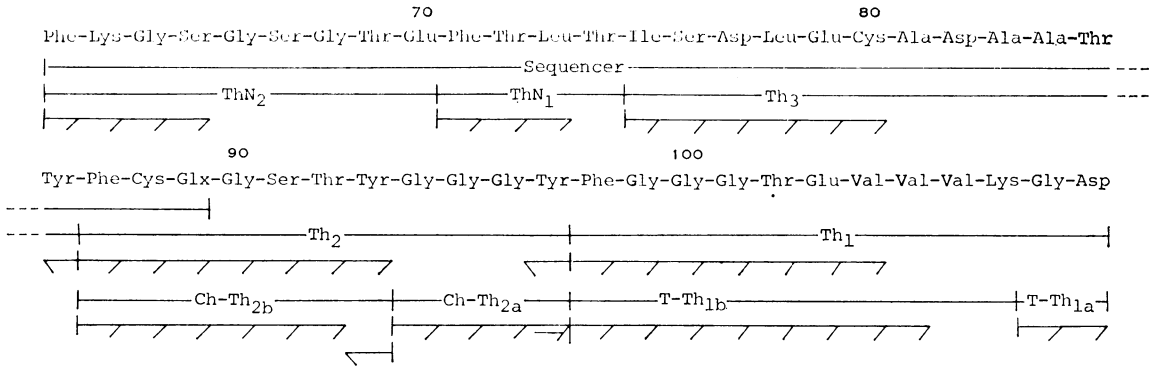

Fig. 6. *Amino acid sequence of the fragment A_3 of L chain BS-1 (residues 62–109)*

For experimental details see the text and Table 2. T, Th and Ch are tryptic, thermolysin and chymotryptic peptides respectively. ⇁ , Amino acid residues identified by the 'dansyl–Edman' procedure; ⇒ identified as dansyl residue without hydrolysis; ↼, identified by carboxypeptidase A or B. Residues identified with the sequencer are also shown.

Table 3. *Amino acid composition and properties of constituent peptides of the N-terminal fragment B of L chain BS-1*

Values are residues/molecule of peptide. Values in parentheses are integral values confirmed by sequence analysis. Chymotryptic peptides, ChT_{3-1}, ChT_{3-2} and ChT_{3-3}, were derived from peptide T_3; and ChT_{4-1} and ChT_{4-2} from peptide T_4, respectively. Mobilities are expressed relative to Asp (= −1.0) or to Arg (= +1.0). Yields are based on 2.25 μmol of starting material (*N*-terminal fragment B).

	Tryptic peptides						Chymotryptic peptides						
Amino acid	T_1	T_2	T_3	T_4	T_5	T_6	Ch-1	Ch-2	ChT_{3-1}	ChT_{3-2}	ChT_{3-3}	ChT_{4-1}	ChT_{4-2}
Lys	1.1	1.2	2.1	—	1.2 (1)	1.1 (1)	1.0	2.1	—	—	2.1 (2)	—	—
Arg	—	—	—	1.2 (1)	—	—	1.1	—	—	—	—	—	0.9 (1)
CmCys*	—	0.7	0.8	—	—	—	—	—	0.7 (1)	—	—	—	—
Asp	1.0	—	0.3	—	—	—	—	—	—	—	—	—	—
Thr	3.9	—	—	1.1 (1)	—	—	1.1	—	—	—	—	1.1 (1)	—
Ser	2.1	2.9	2.8	4.2 (4)	—	—	4.2	—	1.7 (2)	1.1 (1)	—	0.8 (1)	3.1 (3)
Glu	2.0	3.8	5.0	—	1.0 (1)	—	—	3.0	2.1 (2)	—	3.0 (3)	—	—
Pro	2.1	0.2	3.4	—	2.8 (3)	—	—	3.1	—	—	2.9 (3)	—	—
Gly	2.1	1.0	1.9	1.1 (1)	1.3 (1)	—	1.0	1.0	—	1.0 (1)	0.9 (1)	—	1.2 (1)
Ala	0.9	1.9	2.1	2.1 (2)	—	—	2.1	—	1.0 (1)	0.9 (1)	—	1.0 (1)	1.0 (1)
Val	4.7	—	—	1.2 (1)	—	—	0.9	—	—	—	—	—	0.8 (1)
Met	0.9	—	—	—	—	—	—	—	—	—	—	—	—
Ile	1.1	1.0	1.1	—	—	0.9 (1)	—	—	0.9 (1)	—	—	—	—
Leu	—	1.2	1.2	1.0 (1)	—	2.0 (2)	0.9	2.2	—	1.1 (1)	—	1.1 (1)	—
Tyr	—	1.8	2.1	—	—	1.1 (1)	—	—	1.1 (1)	0.9 (1)	—	—	—
Trp	—	+†	+†	—	—	—	—	—	—	+† (1)	—	—	—
Total residues	22	17	23‡	11	6	5	12	11	8	6‡	9	4	7
Mobility at pH6.5	−0.16	0	+0.10	+0.28	+0.37	+0.41	+0.24	+0.54	−0.61	0	—	0	+0.33
Yield (%)	15	10	19	27	9	44	30	32	10	12	19	15	16

* Value not corrected for losses during hydrolysis.
† Measured with Ehrlich reagent.
‡ Tryptophan assumed to be 1 residue/molecule of peptide.

Fig. 6 shows that the sequence of the *N*-terminal 28 residues of fragment A_3 overlaps peptides ThN_2, ThN_1, Th_3 and Th_2 in this order. The peptide Th_1 must therefore be the *C*-terminal peptide of fragment A_3.

Sequence determination of tryptic fragment B

After removal of citraconyl groups, fragment B was digested with trypsin and the mixture fractionated by high-voltage paper electrophoresis at pH6.5. Six peptides T_1–T_6 were isolated; peptides T_2 and T_5

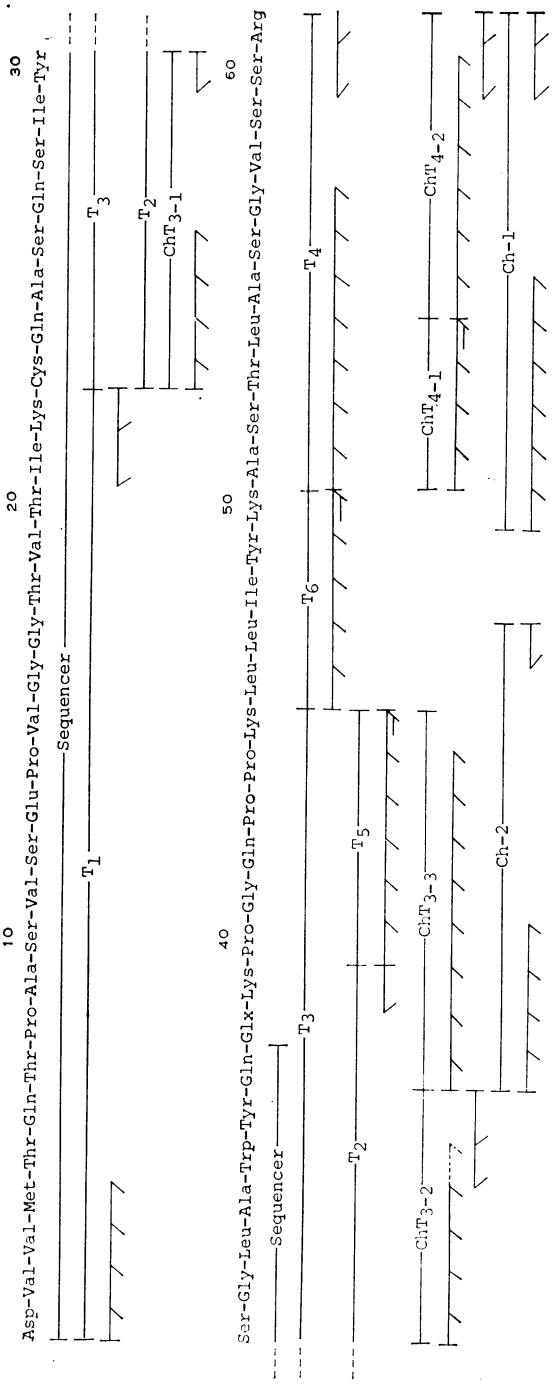

Fig. 7. *Amino acid sequence of the N-terminal fragment B of L chain BS-1* (*residues* 1–61)

For experimental details see the text and Table 3. T and ChT are tryptic and chymotryptic peptides, respectively. Symbols as in Fig. 6.

```
1                                   10                                  20
Asp-Val-Val-Met-Thr-Gln-Thr-Pro-Ala-Ser-Val-Ser-Glu-Pro-Val-Gly-Gly-Thr-Val-Thr-Ile-Lys-Cys-Gln-Ala-

26              30                                  40                                  50
Ser-Gln-Ser-Ile-Tyr-Ser-Gly-Leu-Ala-Trp-Tyr-Gln-Glx-Lys-Pro-Gly-Gln-Pro-Pro-Lys-Leu-Leu-Ile-Tyr-Lys-

51                                  60                                  70
Ala-Ser-Thr-Leu-Ala-Ser-Gly-Val-Ser-Ser-Arg-Phe-Lys-Gly-Ser-Gly-Ser-Gly-Thr-Glu-Phe-Thr-Leu-Thr-Ile-

76              80                                  90                                  100
Ser-Asp-Leu-Glu-Cys-Ala-Asp-Ala-Ala-Thr-Tyr-Phe-Cys-Glx-Gly-Ser-Thr-Tyr-Gly-Gly-Gly-Tyr-Phe-Gly-Gly-

101                                 110                                 120
Gly-Thr-Glu-Val-Val-Val-Lys-Gly-Asp-Pro-Val-Ala-Pro-Thr-Val-Leu-Ile-Phe-Pro-Pro-Ala-Ala-Asp-Gln-Val-

126             130                                 139
Ala-Thr-Gly-Thr-Val-Thr-Ile-Val-Cys-Val-Ala-Asn-Lys-Tyr
```

Fig. 8. *Amino acid sequence of the N-terminal* 139 *residues of L chain BS-*1

The sequence comprises the *N*-terminal fragment B (residues 1–61), fragment A_3 (residues 62–109) and part of the fragment A_1 subjected to automated Edman degradation (residues 110–139).

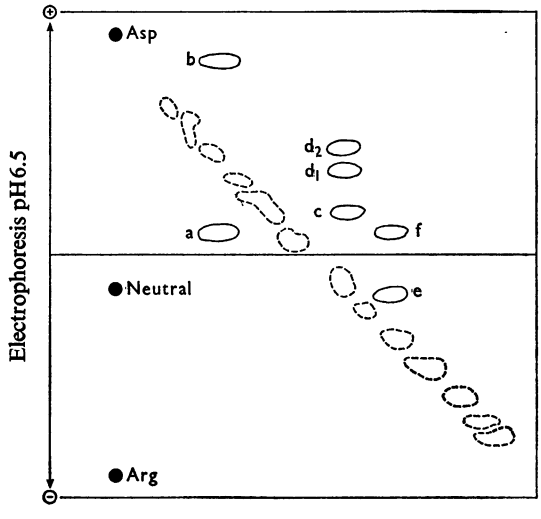

Fig. 9. *Diagonal peptide 'map' at pH*6.5 *of antibody L chain BS-*1

●, Electrophoretic markers. From left to right: cysteine-80- and cysteine-171-containing peptides (b and a); cysteine-23- and cysteine-88-containing peptides (c, d_1 and d_2); cysteine-134- and cysteine-194 containing peptides (f and e). See Table 4 for details.

were repurified by electrophoresis at pH3.5. Their amino acid composition, mobility at pH6.5 and yield are given in Table 3.

Peptide T_1 is a 22-residue peptide and the only one containing the single methionine present in position 4 of fragment B. Its *N*-terminal sequence determined by the 'dansyl–Edman' procedure was Asp-Val-Val-Met. Peptide T_1 is thus the *N*-terminal peptide of fragment B (Fig. 7).

Peptides T_2 and T_3 are the only two radioactive peptides in the digest, Both start with the sequence CmCys-Glx-Ala. Peptide T_3 has 23 residues including 2 lysine and 3 proline residues. Peptide T_2 is shorter by 6 residues and contains only 1 lysine, which is *C*-terminal, but no proline residues. Peptide T_3 was further fragmented with α-chymotrypsin and three peptides, ChT_{3-1}, ChT_{3-2} and ChT_{3-3}, were purified by electrophoresis at pH3.5 and/or 6.5. The amino acid composition of the octapeptide ChT_{3-1} and its partial sequence Cys-Glx-Ala-Ser indicate that it is the *N*-terminal peptide of fragment T_3. The nonapeptide ChT_{3-3}, rich in proline and lysine residues, must be the *C*-terminal peptide of peptide T_3: eight cycles of Edman degradation established the sequence Glx-Glx-Lys-Pro-Gly-Gln-Pro-Pro-Lys (Fig. 7). The neutral hexapeptide ChT_{3-2} contains tryptophan and extends from residues 31–36. The identification of tryptophan in the fifth step of 'dansyl–Edman' degradation was dubious. However, carboxypeptidase A digestion suggested the *C*-terminal sequence to be Trp-Tyr. Tryptophan was identified as the phenylthiohydantoin derivative at step 35 of the automated Edman degradation of fragment B. The sequence of the *N*-terminal 37 residues of fragment B, determined by the protein sequencer, overlaps peptides T_1, T_2 and T_3 and thus enables their alignment (Fig. 7).

Peptide T_4 is the only arginine-containing peptide of the digest and should therefore be the *C*-terminal peptide of fragment B. After chymotrypsin digestion, peptide T_4 gave rise to the tetrapeptide ChT_{1-4} and to the heptapeptide ChT_{4-2}. Their sequence is given in Fig. 7.

Table 4. *Peptic peptides isolated from L chain BS-1 by diagonal electrophoresis*

Mobilities at pH 6.5 are expressed relative to Asp = −1. A loss of about 50% of the tyrosine residues in cysteic acid-containing peptides d_1, d_2, e and f was found as a result of performic acid oxidation. The numbered positions of the cysteine residues 23, 80, 88 and 134 are deduced from a comparison of the amino acid composition and partial *N*-terminal sequence of the cysteic acid-containing peptides with the extended sequence of L chain BS-1 (Fig. 9). The positions of the cysteine residues 171 and 194 are assigned by homology with human κ chains (Putnam *et al.*, 1967). The *N*-terminal sequence was established by the dansyl–Edman procedure.

Position of cystine residues	Peptic peptide	Mobility at pH 6.5	Amino acid composition	*N*-terminal sequence
23	c	−0.20	(Ile,Lys,Cys,Glx,Ala,Ser)	Ile-Lys-Cys
88	d_1	−0.43	($Tyr_{0.5}$,Phe,Cys,Glx,Gly,Ser,Thr)	Tyr-Phe-Cys
88	d_2	−0.47	($Tyr_{0.5}$,Phe,Cys,Glx,Gly,Ser,Thr)	Tyr-Phe-Cys
80	b	−0.91	(Glx,Cys,Asx,Ala,Ala,Ala,Thr)	Glx-Cys-Ala
171	a	−0.11	(Ser,Lys,Thr,Pro,Glx,Asx,Ser,Ala,Asx,Cys,Thr)	Ser-Lys-Thr
134	f	−0.13	(Ile,Val,Cys,Val,Ala,Asx,Lys,$Tyr_{0.5}$,Phe,Pro,Asx,Val,Thr)	Ile-Val-Cys-Val
194	e	0	(Thr,Ser,Thr,Glx,$Tyr_{0.5}$,Asx,Ser,His,Lys,Glx,$Tyr_{0.5}$,Thr,Cys, Lys,Gly,Thr,Val)	Thr

The basic peptide T_5 contains 3 proline residues and has the sequence Pro-Gly-Gln-Pro-Pro-Lys. It is assigned as the *C*-terminal hexapeptide of fragment T_3. This suggests that the tryptic peptide T_2 arose from peptide T_3 by partial tryptic cleavage of the Lys_{39}–Pro_{40} bond (Fig. 7).

Finally, the basic pentapeptide T_6 was isolated in good yield and has the sequence Leu-Leu-Ile-Tyr-Lys. Two peptides, Ch-1 and Ch-2, which were isolated from a chymotryptic digest of fragment B, overlap peptide T_6, which therefore should extend in positions 46–50 between peptides T_3 and T_4. The sequence determination of the *N*-terminal fragment B is summarized in Fig. 7.

The amino acid composition determined for fragments B and A_3, which represent the entire V domain, is in good agreement with that computed from the sequence (Table 1). The amino acid sequence of the *N*-terminal 139 residues of L chain BS-1 is shown in Fig. 8.

Intrachain disulphide bridges of L chain BS-1

L chain BS-1 contains 6.6 residues of *S*-carboxymethylcysteine/molecule, which indicates that this protein belongs to the K_B type, characterized by three disulphide bridges (Rejnek *et al.*, 1969). Poulsen *et al.* (1972) have demonstrated that the third, i.e. the 'extra' disulphide bridge connects the V and C domains. However, the position of the half-cystine within the V region has not yet been reported. The sequence of the V region (the present paper) and the partial sequence data from the C region (Appella *et al.*, 1971; Strosberg *et al.*, 1972) enabled us to study the pairing of the cysteine residues. Diagonal peptide 'mapping' was done at pH 6.5 with a peptic digest of the L chain. After performic acid oxidation and electrophoresis in the second direction, three sets of two cysteic acid-containing peptides (a and b, c and d_1+d_2, and e and f respectively) were separated as outlined in Fig. 9 and characterized as summarized in Table 4.

Three cysteic acid-containing peptides, c, d_1 and d_2, constitute the first intrachain disulphide bridge 23–88 of the V region. Peptides d_1 and d_2 are identical in *N*-terminal sequence and probably in amino acid composition; they differ however slightly in electrophoretic mobility. Peptides b and a form the 'extra' disulphide bond of the rabbit K_B chain: 1 cysteine residue in one of these can be convincingly placed at position 80 in the sequence of BS-1 L chain (Fig. 8), whereas its partner is the cysteine-171 as deduced by homology with human and rabbit κ-chain sequences from the C region. The amino acid composition and partial sequence of the cysteic acid-containing peptides f and e indicate that they form the disulphide bridge 134–194 of the constant region in agreement with the results of Appella *et al.* (1971); the position of the cysteine residue-134 is deduced from the BS-1 sequence (Fig. 8) and that of the cysteine-194 is based on homology with human κ chains (Putnam *et al.*, 1967).

Discussion

L chain BS-1 contains 2 arginine residues. One of these is the fourth residue from the *C*-terminal end (Appella *et al.*, 1969) and the other is assumed to be located at position 61 in the V region (Freedlender & Haber, 1972). This suggested that tryptic cleavage restricted to the arginine residues would offer a method for obtaining large V-region fragments suitable for automated Edman degradation. Indeed, two large arginine-containing peptides, A (residues

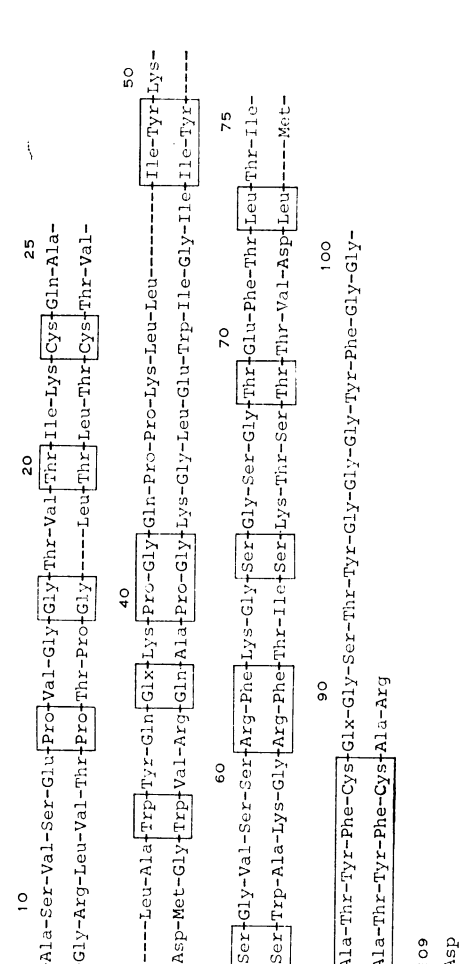

Fig. 10. *Comparison of the V region of L chain BS-1 with the V region of anti-pneumococcal H chains*

Identical residues are framed in boxes. Deletions (----) are introduced to maximize homology. The V_H region sequence of residues 1–65 is derived from the anti-pneumococcal type III antibody BS-5 (Jaton & Braun, 1972); the sequence of residues 65–75 is from a_1 pooled normal H chains (Mole *et al.*, 1971) and that of residues 76–90 is from the corresponding H chain BS-1 (Jaton *et al.*, 1973).

62–211) and B (residues 1–61), were isolated in high yield. Fragment A was further cleaved at the acid-labile Asp_{109}–Pro_{110} bond (Fraser *et al.*, 1972) into two major peptides, A_1 and A_3. The entire V region is made up of only two large peptides, B and A_3, and fragment A_1 contains most of the C region (Fig. 5). Automated Edman degradation of peptides B and A_3 enabled us to determine 60% of the sequence (Fig. 2) and this knowledge is very valuable for ordering small tryptic, chymotryptic and thermolysin peptides. It should be pointed out that the procedures used in this work required only 4μmol of L chain, an amount which is substantially smaller than that used with the traditional sequencing methodology.

Homogeneity of the V region of the L chain is shown by (1) the unique sequence determined with the protein sequencer in sections 1–37 and 62–89, (2) the high yield of the *N*-terminal fragment B (82%) and of peptide A_3 (65%), and (3) the recoveries of small peptides isolated from the hypervariable sections of fragments A_3 and B by paper electrophoresis, which compare favourably with those reported for human and mouse myeloma κ chains (Tables 2 and 3). However, minor variant peptides, if present at the 10% value, might have escaped detection by the techniques used here.

The observation of the partial tryptic cleavage at the Lys_{39}–Pro_{40} bond is somewhat unexpected (Fig. 7). That this cleavage is a result of the acid treatment of fragment B during decitraconylation is unlikely, as only one *N*-terminal sequence could be determined when a portion of decitraconylated fragment B was subjected to automated Edman degradation (Fig. 2). The partial tryptic cleavage of this bond was also noted in the study of two other rabbit L chains (J.-C. Jaton, unpublished work) and was reported to occur, in homologous positions, in murine κ chains (McKean *et al.*, 1973).

When compared with the basic sequences of human κ chains (Milstein & Pink, 1970), the V-region sequence of L chain BS-1 exhibits 66%, 53% and 54% homology respectively with K_I, K_{II} and K_{III} sequences (the comparison does not include hypervariable positions). Sections 20–30, 34–41, 44–49 and 60–88 of L chain BS-1 are almost identical with their human counterparts. Thus L chain BS-1 appears to be more similar to V_{KI} prototype sequence than to V_{KII} and to V_{KIII} basic sequences. The degree of homology between the L chain V region subgroups of different species is similar to that found between the H chain V regions of various species (Ray & Cebra, 1972; Kehoe & Capra, 1972; Bourgois *et al.*, 1972; Jaton & Braun, 1972). The V region of L chain BS-1 was compared with the V region of anti-pneumococcal H chains (Fig. 10). Homology of these V regions is evident, as of 90 residues there are 28 identical residues in homologous positions. This confirms the original finding of Edelman (1970) on protein Eu and

suggests that V-region sequences from both H and L chains evolved from a common precursor.

On the basis of the great sequence variability observed in the *N*-terminal 20–30 positions of rabbit L chains, the existence of at least 6 V_K region subgroups was proposed (Braun & Jaton, 1973). Anti-pneumococcal and streptococcal L chain *N*-terminal sequences obtained to date distribute between several subgroups as do also anti-hapten L chains (Appella *et al.*, 1971; Freedman *et al.*, 1972). This suggests that there is no obligatory association between antibody specificity and L chain subgroups, even though preferential expression of a given subgroup has been noted for other antigens (Potter *et al.*, 1970; Capra *et al.*, 1972). Further, the L chains of a parent and of an offspring rabbit immunized with the same bacterial vaccine were found identical within their *N*-terminal 22 amino acid residues. These and additional data suggest preferential expression of inherited V-region genes for the synthesis of anti-polysaccharide antibodies (Braun & Jaton, 1973).

Among mammalian species which have been studied so far, rabbit κ chains are unique in possessing three intrachain disulphide bridges (Rejnek *et al.*, 1969). One of them has been shown to link V and C domains (Poulsen *et al.*, 1972). The interdomain connecting bond is formed by a half-cystine in position 80 and its partner is found in position 171. These results (Fig. 9, Table 4) confirm and extend those recently reported by Lamm & Frangione (1972). The 'domain' hypothesis (Edelman *et al.*, 1969) predicts that V and C regions each containing one disulphide bond are folded independently as compact domains linked by loose connecting regions. This is quite consistent with the X-ray crystallographic model of a human Fab' fragment (Poljak *et al.*, 1972). As the disulphide bonds are known to be an important factor for maintaining and stabilizing the conformation of polypeptide chains, one can wonder whether alteration in the number and in the arrangement of the disulphide bridges would create a distortion of the three-dimensional structure of the molecule. The presence of the interdomain-connecting disulphide bond 80–171 suggests that the region around cysteine-80 should come very close to the cysteine-171 region. This indeed was demonstrated by the X-ray analysis of a Bence-Jones protein dimer which lacks this disulphide bond (Schiffer *et al.*, 1973). The inspection of the X-ray model at 0.28nm (2.8Å) resolution (Poljak *et al.*, 1973) suggests that the presence of that bridge would not impose a conformational constraint on the folding of the V and C domains of the L chain.

The presence of two clusters of 3 glycine residues in a row in positions 94–96 and 99–101 is particularly striking. The glycine residues in positions 99–101 are essentially invariant residues in human L chains. They may provide flexibility of the polypeptide chain to allow a better fit for the V_L region in contact with V_H region, once an antigenic determinant reacts with the antibody-combining site (Wu & Kabat, 1970). Glycine residues at positions 94–96 are unique to this protein and are located in the hypervariable area. They may therefore be related to the specificity of this antibody. In other antibody L chains of similar but not necessarily identical specificity, glycine residues 94–96 are substituted by other residues, whereas those in positions 99–101 are invariant. (Jaton, 1974, and unpublished work). The X-ray-diffraction data, in conjunction with amino acid-sequence data, are expected to reveal which amino acid substitutions are permissible within the V regions without markedly altering the three-dimensional structure of the antibody molecule.

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