Biochem. J. (1975) **147**, 235–247
Printed in Great Britain

Comparison of the Amino Acid Sequences of the Variable Domains of Two Homogeneous Rabbit Antibodies to Type III Pneumococcal Polysaccharide

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(*Received* 30 *October* 1974)

The amino acid sequences of the V (variable) regions of the H (heavy) and L (light) chains derived from rabbit antibody K-25, specific for type III pneumococci, were determined; this is the second homogeneous rabbit antibody besides antibody BS-5 whose complete sequence of the V domain has been established (Jaton, 1974*d*). The V regions of L chains BS-5 and K-25 (both of allotype b_4) differ from each other by 19 amino acid residues; 11 of these 19 substitutions are located within the three hypervariable sections of the V region. On the basis of seven amino acid differences within the *N*-terminal 28 positions, it is suggested that L chain K-25 belongs to a different subgroup of rabbit κ chains than L chain BS-5. H chain K-25 (allotype a_2) differs from another H chain of the same allotype by one amino acid substitution within the *N*-terminal 70 positions in addition to interchanges occurring in the first two hypervariable sections. H chain K-25 was compared with H chain BS- 5(allotype a_1) and with the known V-region rabbit sequences. Allotype-related differences between a_1, a_2 and a_3 chains appear to occur within the *N*-terminal 16 positions and possibly in scattered positions throughout the V-region. In the hypervariable positions, variability between the two antibodies is remarkably more pronounced within the third hypervariable section of both H and L chains than within the first two.

The amino acid sequence of the variable* (V) regions of both heavy (H) and light (L) chains (V domain) derived from the homogeneous anti-pneumococcal antibody BS-5 has been completed (Jaton, 1974*d*). The V domain of the immunoglobulin molecule carries the antibody-binding site as well as important genetic markers, e.g. the allotypes of the group *a* (Todd, 1963; Todd & Inman, 1967; Wilkinson, 1969) and the idiotypic determinants (Wells *et al.*, 1973).

Homogeneous antibodies to type III pneumococcal polysaccharide can serve as a useful model to study several parameters involved in the interactions between the antibody and its ligand, for example the role of the hypervariable sections of the V domain in specifying the antibody activity, the affinity for the ligand, and the geometry of the active site. Comparison of the amino acid sequences of several V domains of antibodies to the same polysaccharide antigen should help define the structural basis of antibody specificity.

This report describes the primary structure of the V domain of another homogeneous antibody designated K-25 directed against type III pneumococcal polysaccharide. Its H chain, which carries the allotype a_2, provides a direct comparison with the H chain of antibody BS-5, of allotype a_1. The partial *N*-terminal amino acid sequence of H chain K-25 has been reported (Jaton & Haimovich, 1974).

* 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; CmCys and $\overline{\text{Glu}}$ (in amino acid sequences and Tables), *S*-carboxymethylcysteine and pyrrolidonecarboxylic acid acid respectively; V_{HI}, V_{HII} and V_{HIII}, subgroups I, II and III respectively of V regions of H chains; V_{KI}, V_{KII} and V_{KIII}, subgroups I, II and III respectively of V regions of κ L chains; the nomenclature for subgroups of human κ chains is that used by Milstein & Pink (1970).

Experimental

Materials

Enzymes, chemicals and reagents used for the characterization of the peptides and for the sequence determination were described previously (Jaton, 1974*a*).

Methods

Antibody production and purification. Rabbit K-25 was immunized with type III pneumococcal vaccine (Kimball *et al.*, 1971) and the antibody isolated as

described previously (Braun & Jaton, 1973). It carries a_2b_4 allotypic markers. This was kindly determined by Dr. A. S. Kelus, Basel Institute for Immunology.

Isolation of peptides. H and L chains were separated after mild reduction and alkylation and large arginine-containing peptides were produced from the reduced–alkylated and citraconylated L chain by trypsin digestion, and isolated by Sephadex chromatography (Jaton, 1974*a*). The removal of citraconyl groups was performed as described previously (Jaton, 1974*b*). Cleavage of the labile Asp-Pro bond between V_L and C_L regions was done as recommended by Fraser *et al.* (1972). The preparation of the *N*-terminal half of the H chain (fragment C1) from a CNBr digest of the H chain, and the complete reduction and alkylation with iodo[2-^{14}C]acetic acid, were performed as described previously (Jaton & Braun, 1972). The citraconylation of large CNBr fragments from fragment C1 and the purification of the arginine-containing peptides have been described (Jaton, 1974*d*).

Analytical methods. Preparative high-voltage paper electrophoresis, detection of peptides, enzymic digestions, radioautography, amino acid analyses and the determination of sequences of small peptides by the 'dansyl–Edman' procedure were performed as described previously (Jaton, 1974*a*).

Amino acid-sequence analysis of large peptides was performed in the Beckman Sequencer model 890B, equipped with an undercut cup and N_2 flush, by using either the Quadrol buffer or the volatile *NN*-dimethylbenzylamine buffer (Hermodson *et al.*, 1972). The volatile buffer programme has been described in detail elsewhere (Jaton, 1974*c*).

The determination of the sequence of lysine-containing peptides by using 4-sulphophenyl isothiocyanate to minimize losses of peptides (Braunitzer *et al.*, 1971) and the identification of phenylthiohydantoin derivatives were carried out as previously described (Jaton, 1974*d*).

Results

Primary structure of the V region of H chain K-25

Cleavage of H chain K-25 with CNBr. The *N*-terminal half of H chain K-25 (CNBr fragment C1) has been shown to contain two internal methionine residues, one at position 33 and the other at position 78, thus yielding three fragments on CNBr cleavage accounting for residues 1–33, 34–78 and 79–253 (Jaton & Haimovich, 1974). The components of the digest were separated into two fractions, A and B, by gel filtration on a column of Sephadex G-100 in the presence of 5M-guanidine hydrochloride (Jaton & Haimovich, 1974). Fraction A was shown to comprise the peptides containing residues 1–33 and 79–253 linked together by the disulphide bridge between residues 21 and 91, whereas fraction B contained the peptide with residues 34–78 free of half-cystine residues (Table 1). Fragment B was obtained in low yield (25%), as it interacts strongly with fragment C1 by non-covalent forces (Jaton *et al.*, 1973).

Characterization of large peptides from the reduced and alkylated fraction A. The *N*-terminal 33-residue peptide of H chain K-25 was isolated by chromatography on a column of Sephadex G-75 in 5M-guanidine hydrochloride after reduction and alkylation of fraction A with [2-^{14}C]iodoacetate (Jaton & Braun, 1972). It was recovered in 55% yield (Jaton & Haimovich, 1974).

The reduced and alkylated fraction A, devoid of the *N*-terminal 33-residue peptide, was then citraconylated and digested with trypsin; the digest was loaded on a column of Sephadex G-50, equilibrated with 5M-guandidine hydrochloride at pH 5.5, and three fractions designated CT-A_1, CT-A_2 and CT-A_3 according to the order of elution, were obtained. The profile was similar to that described for H chain BS-5 (see Fig. 2 of Jaton, 1974*d*). Fractions CT-A_1 and CT-A_2, which were cross-contaminated, were further purified by ion-exchange chromatography (in the citraconylated form) on DEAE-Sephadex A-25 (Cebra *et al.*, 1968). Fragment CT-A_1 contains one homoserine residue, which was shown to be the *C*-terminal residue by carboxypeptidase A digestion, and no arginine residue; its amino acid composition was identical with that shown for the homologous peptide derived from H chain BS-5 (Jaton, 1974*d*) and for the *C*-terminal peptide T_2 of fragment C1 derived from pooled H chains (Cebra *et al.*, 1968). This fragment is the *C*-terminal peptide of the constant domain C_{H1} and it was therefore not characterized further.

The other large citraconyl peptide, CT-A_2, had arginine as *C*-terminal and glycine as *N*-terminal amino acid. Its amino acid composition is presented in Table 1.

The third fraction, CT-A_3, was decitraconylated (Jaton, 1974*b*), freeze-dried and desalted on a column of Sephadex G-25 equilibrated in 0.02M-NH_3. A portion of this fraction was analysed by high-voltage paper electrophoresis at pH 6.5, and revealed one major peptide, designated T-A_3, which was radioactive and accounted for most of the radioactivity of the fraction. A few peptides which remained at the origin were present in lower yield. Tryptic digestion of the eluted material which did not move on paper yielded smaller peptides which could be shown to originate from the *N*-terminal 33-residue peptide and from fragment B. The amino acid composition and the electrophoretic mobility of the major peptide, T-A_3, is reported in Table 1.

Table 1. *Amino acid composition of selected peptides from the V region of H chain K-25*

Values are residues/molecule of peptide. Values in parentheses are integral values confirmed by sequence analysis. Peptides T_{B-3}, T_{B-2} and T_{B-N3} are derived from fragment B and the chymotryptic peptide T-A_3Ch_1 is from peptide T-A_3. Mobilities are expressed relative to Asp (= −1.0) or to Arg (= +1.0). N.D., not determined.

		Tryptic peptides					
Amino acid	Fragment B	T_{B-2}	T_{B-3}	T_{B-N3}	T-A_3	CT-A_2	Chymotryptic peptide T-A_3Ch_1
Lys	2.3	—	—	—	—	3.4	—
His	—	—	—	—	—	0.8	—
Arg	3.1	1.0 (1)	1.0 (1)	—	1.1 (1)	0.9	1.0
CmCys	—	—	—	—	0.8 (1)	2.4	0.7 (1)
Asp	2.0	—	—	1.9 (2)	2.0 (2)	4.0	—
Thr	6.1	2.0 (2)	—	1.8 (2)	2.6 (3)	11.2	—
Ser	5.6	1.1 (1)	1.1 (1)	1.2 (1)	1.3 (1)	8.2	—
Glu	2.1	—	—	—	1.0 (1)	1.5	—
Pro	0.9	—	—	—	—	6.5	—
Gly	3.9	—	—	—	—	9.2	—
Ala	4.1	—	—	—	2.6 (3)	3.2	0.9 (1)
Val	2.7	—	—	0.9 (1)	—	10.4	—
Ile	3.2	1.0 (1)	—	—	—	—	—
Leu	2.0	—	—	1.0 (1)	1.0 (1)	8.8	—
Tyr	3.2	—	—	—	1.0 (1)	2.6	—
Phe	—	—	—	—	0.9 (1)	1.2	1.0 (1)
Hse	0.7	—	—	0.7 (1)	—	—	—
Trp	3.0*	—	—	—	—	+†	—
Total residues	44.3	5	2	8	15	74.3	4
Mobility at pH6.5	N.D.	+0.44	+0.80	0	−0.33	N.D.	0
Yield (%)	25‡	42§	24§	29§	31‡	19‡	N.D.

* Tryptophan determined by sequencer analysis.
† Tryptophan determined by Ehrlich reagent.
‡ On the basis of 2500 nmol of fragment C1.
§ On the basis of 225 nmol of fragment B (residues 34–78).

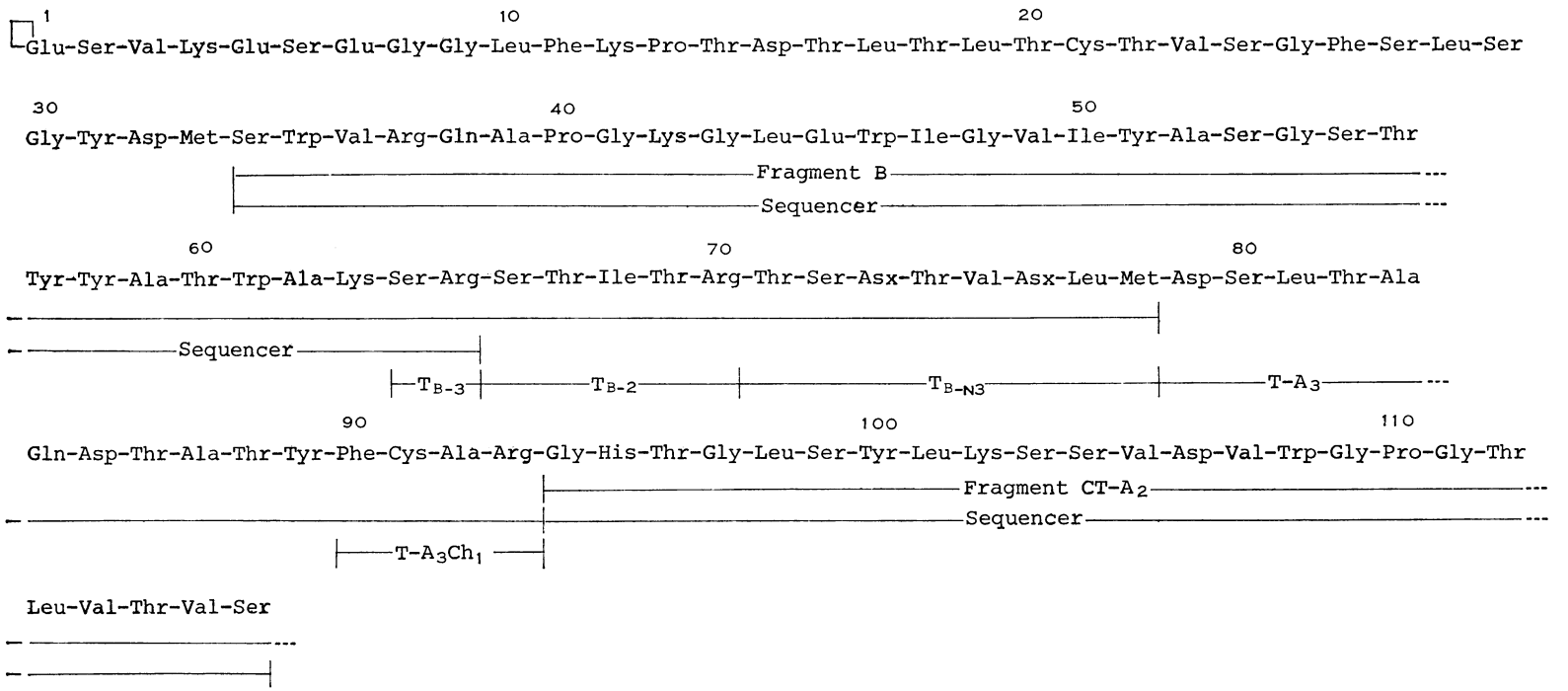

Fig. 1. *Amino acid sequence of the variable region of rabbit H chain K-25*

Residues 34–65 and 94–117 were identified by the sequencer. T and Ch are tryptic and chymotryptic peptides respectively; see Fig. 3 and Table 1. For details about the sequence of residues 1–33, see Jaton & Haimovich (1974).

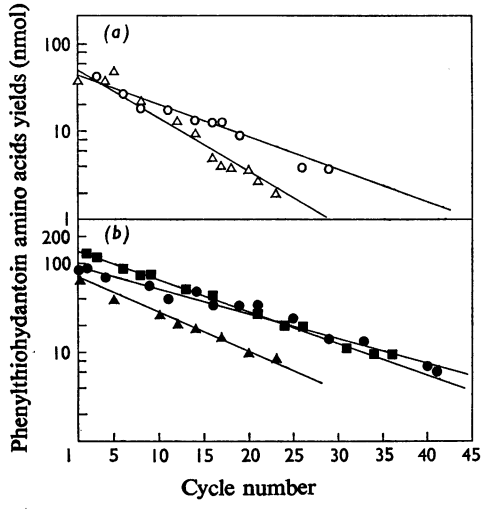

Fig. 2. *Quantitative yields of phenylthiohydantoin amino acids obtained at selected cycles of automated Edman degradations*

Yields are computed by comparing peak heights of sample with relevant standard derivatives by g.l.c. (*a*) ○, Fragment B of H chain K-25; △, fragment CT-A_2 of H chain K-25. The average repetitive yields were 90 and 92% for fragment CT-A_2 and fragment B respectively. (*b*) ●, L chain K-25; ▲, fragment A of L chain K-25; ■, fragment A_1 of L chain K-25. The average repetitive yields were 92, 93 and 94% for fragment A, fragment A_1 and L chain K-25 respectively.

Sequence of the N-terminal 33-residue peptide. This has already been reported together with the sequence of the *N*-terminal 15 residues of fragment B (Jaton & Haimovich, 1974).

Sequence of fragment B. Fragment B (220nmol) was subjected to 32 successful cycles of automated Edman degradation by using the volatile *NN*-dimethylbenzylamine buffer and 4-sulphophenyl isothiocyanate to prevent mechanical losses of peptide during the degradations. A unique sequence was found (Fig. 1); the yields of phenylthiohydantoin derivatives as selected steps in the degradation of fragment B is plotted against the number of steps performed (Fig. 2*a*). The *C*-terminal sequence of fragment B was obtained after isolation and characterization of tryptic peptides derived from fragment B. The amino acid analyses of peptides T_{B-3}, T_{B-2} and T_{B-N3} are described in Table 1, and their sequences in Fig. 3. As peptide T_{B-N3} contains one homoserine residue/molecule, it is the *C*-terminal octapeptide of fragment B. Peptide T_{B-3} is a dipeptide, Ser-Arg, which corresponds to the last two residues in the sequence of fragment B determined by the sequencer (cycles 31–32). The pentapeptide T_{B-2}, Ser-Thr-Ile-

		Position in the sequence
Tryptic peptides		
$T\text{-}A_3$	Asp-Ser-Leu-Thr-Ala-Gln-Asp-Thr-Ala-Thr-Tyr-(Phe-CmCys)-Ala-Arg	79–93
T_{B-2}	Ser-Thr-Ile-Thr-Arg	66–70
T_{B-3}	Ser-Arg	64–65
T_{B-N3}	Thr-Ser-Asx-Thr-Val-Asx-Leu-Hse	71–78
Chymotryptic peptide		
$T\text{-}A_3Ch_1$	Phe-CmCys-Ala-Arg	90–93

Fig. 3. *Sequences of some peptides from fragment C1 of H chain K-25*

The amino acid sequence was established by the dansyl-Edman procedure (—›) and by digestion with carboxypeptidases A and B (—). ⇀, Dansyl residue identified without hydrolysis. The amino acid compositions and mobilities of the peptides are given in Table 1.

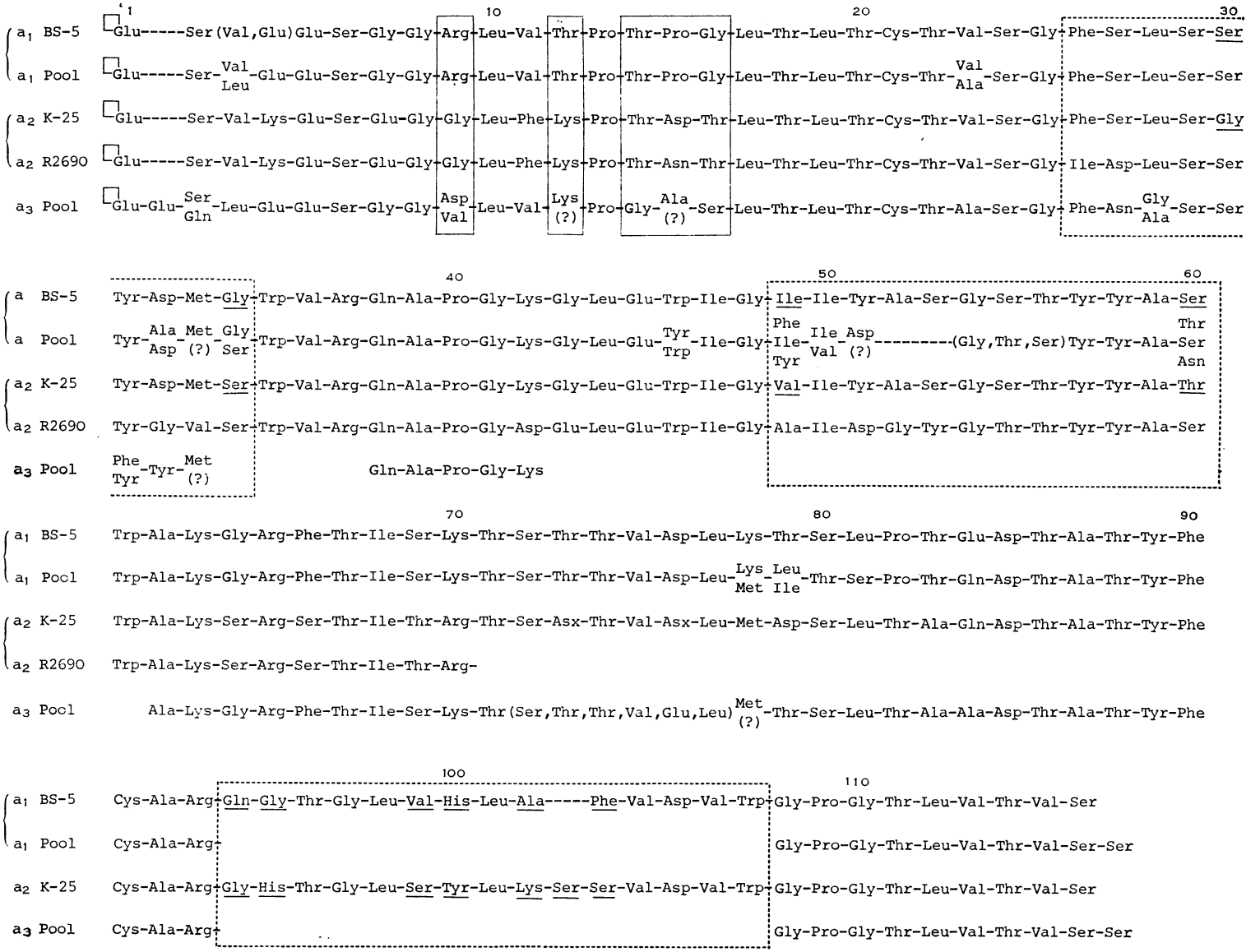
1 10 20 30
a1 BS-5 Glu-----Ser(Val,Glu)Glu-Ser-Gly-Gly-Arg-Leu-Val-Thr-Pro-Thr-Pro-Gly-Leu-Thr-Leu-Thr-Cys-Thr-Val-Ser-Gly-Phe-Ser-Leu-Ser-Ser
a1 Pool Glu-----Ser-Val/Leu-Glu-Glu-Ser-Gly-Gly-Arg-Leu-Val-Thr-Pro-Thr-Pro-Gly-Leu-Thr-Leu-Thr-Cys-Thr-Val/Ala-Ser-Gly-Phe-Ser-Leu-Ser-Ser
a2 K-25 Glu-----Ser-Val-Lys-Glu-Ser-Glu-Gly-Gly-Leu-Phe-Lys-Pro-Thr-Asp-Thr-Leu-Thr-Leu-Thr-Cys-Thr-Val-Ser-Gly-Phe-Ser-Leu-Ser-Gly
a2 R2690 Glu-----Ser-Val-Lys-Glu-Ser-Glu-Gly-Gly-Leu-Phe-Lys-Pro-Thr-Asn-Thr-Leu-Thr-Leu-Thr-Cys-Thr-Val-Ser-Gly-Ile-Asp-Leu-Ser-Ser
a3 Pool Glu-Glu-Ser/Gln-Leu-Glu-Glu-Ser-Gly-Gly-Asp/Val-Leu-Val-Lys/(?)-Pro-Gly-Ala/(?)-Ser-Leu-Thr-Leu-Thr-Cys-Thr-Ala-Ser-Gly-Phe-Asn-Gly/Ala-Ser-Ser
40 50 60
a BS-5 Tyr-Asp-Met-Gly-Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Ile-Gly-Ile-Ile-Tyr-Ala-Ser-Gly-Ser-Thr-Tyr-Tyr-Ala-Ser
a Pool Tyr-Ala/Asp-Met/(?)-Gly/Ser-Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Gly-Leu-Glu-Tyr/Trp-Ile-Gly-Phe/Ile-Ile/Val-Asp/(?)---------(Gly,Thr,Ser)Tyr-Tyr-Ala-Thr/Ser/Asn
a2 K-25 Tyr-Asp-Met-Ser-Trp-Val-Arg-Gln-Ala-Pro-Gly-Lys-Gly-Leu-Glu-Trp-Ile-Gly-Val-Ile-Tyr-Ala-Ser-Gly-Ser-Thr-Tyr-Tyr-Ala-Thr
a2 R2690 Tyr-Gly-Val-Ser-Trp-Val-Arg-Gln-Ala-Pro-Gly-Asp-Glu-Leu-Glu-Trp-Ile-Gly-Ala-Ile-Asp-Gly-Tyr-Gly-Thr-Thr-Tyr-Tyr-Ala-Ser
a3 Pool Phe/Tyr-Tyr-Met/(?) Gln-Ala-Pro-Gly-Lys
70 80 90
a1 BS-5 Trp-Ala-Lys-Gly-Arg-Phe-Thr-Ile-Ser-Lys-Thr-Ser-Thr-Thr-Val-Asp-Leu-Lys-Thr-Ser-Leu-Pro-Thr-Glu-Asp-Thr-Ala-Thr-Tyr-Phe
a1 Pool Trp-Ala-Lys-Gly-Arg-Phe-Thr-Ile-Ser-Lys-Thr-Ser-Thr-Thr-Val-Asp-Leu-Lys/Met-Leu/Ile-Thr-Ser-Pro-Thr-Gln-Asp-Thr-Ala-Thr-Tyr-Phe
a2 K-25 Trp-Ala-Lys-Ser-Arg-Ser-Thr-Ile-Thr-Arg-Thr-Ser-Asx-Thr-Val-Asx-Leu-Met-Asp-Ser-Leu-Thr-Ala-Gln-Asp-Thr-Ala-Thr-Tyr-Phe
a2 R2690 Trp-Ala-Lys-Ser-Arg-Ser-Thr-Ile-Thr-Arg-
a3 Pool Ala-Lys-Gly-Arg-Phe-Thr-Ile-Ser-Lys-Thr(Ser,Thr,Thr,Val,Glu,Leu)Met/(?)-Thr-Ser-Leu-Thr-Ala-Ala-Asp-Thr-Ala-Thr-Tyr-Phe
100 110
a1 BS-5 Cys-Ala-Arg-Gln-Gly-Thr-Gly-Leu-Val-His-Leu-Ala-----Phe-Val-Asp-Val-Trp-Gly-Pro-Gly-Thr-Leu-Val-Thr-Val-Ser
a1 Pool Cys-Ala-Arg- Gly-Pro-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser
a2 K-25 Cys-Ala-Arg-Gly-His-Thr-Gly-Leu-Ser-Tyr-Leu-Lys-Ser-Ser-Val-Asp-Val-Trp-Gly-Pro-Gly-Thr-Leu-Val-Thr-Val-Ser
a3 Pool Cys-Ala-Arg- Gly-Pro-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser

Fig. 4.

Thr-Arg, is placed between peptides T_{B-3} and T_{B-N3} (Fig. 1) by homology with the partial sequence of another antibody H chain of the same allotype a_2 (Fleischman, 1973). By homology with the partial sequence of rabbit H chains (Mole *et al.*, 1971; Fleischman, 1971; Jaton, 1974*d*), the sequence of fragment B extends between residues 34 and 78.

Partial N-terminal sequence of fragment CT-A_2. Decitraconylated fragment CT-A_2 (200nmol) was first treated with 4-sulphonyl isothiocyanate and degraded by using the volatile buffer programme in the protein sequencer. The clear-cut identification of the first *N*-terminal 24 residues is reported in Fig. 1. The repetitive yields during selected cycles of degradation are presented in Fig. 2(*a*). No evidence for heterogeneity could be detected in that portion of fragment CT-A_2. The sequence of fragment CT-A_2 from positions 13–24 is identical with the rabbit a_1 and a_3 pooled H chain sequences of the constant region (L. E. Mole, unpublished work; Fig. 4) and homologous to the conservative human sequences of the beginning of the C_{HI} domain (Capra & Kehoe, 1974). Fragment CT-A_2 can therefore with confidence be placed between positions 94 in the V region (Fig. 1) and 170 in the C_{HI} region (Fruchter *et al.*, 1970), i.e. it comprises the third hypervariable section of the rabbit H chain in its *N*-terminal part and the beginning of the C_{HI} domain in its *C*-terminal part.

Sequence of peptide T-A_3. This was established by the dansyl–Edman procedure; digestion with carboxypeptidases A and B revealed arginine and alanine in low yield. A chymotryptic digest of peptide T-A_3 released the *C*-terminal radioactive tetrapeptide Phe-CmCys-Ala-Arg. The amino acid composition and the determination of the sequence of peptide T-A_3 are shown in Table 1 and Fig. 3 respectively. By homology with the known sequences of rabbit H chain V regions (Mole *et al.*, 1971; Jaton, 1974*d*), peptide T-A_3 occupies residue positions 79–93 (Fig. 1).

The amino acid sequence of the V region of H chain K-25, summarized in Fig. 1, comprises successively the sequences of the *N*-terminal 33-residue peptide, of the CNBr fragment B, of peptide T-A_3 and of the *N*-terminal part of fragment CT-A_2.

Primary structure of the V region of L chain K-25

Characterization of large citraconyl fragments. The amino acid composition of L chain K-25 indicates the presence of only two arginine residues per molecule (not shown). L chain K-25 (4μmol) was therefore reduced and alkylated with iodo[2-^{14}C]-acetate, citraconylated, digested with trypsin and the digest loaded on a Sephadex G-50 column equilibrated in 5M-guanidine hydrochloride, pH5.5. Two large fragments, A and B, were obtained. The *N*-terminal sequence of the decitraconylated fragment B determined by the dansyl–Edman method, Ala-Val-Glu-Leu, is identical with that found for the *N*-terminal portion of the intact L chain (Braun & Jaton, 1973). The amino acid composition of fragment B indicates that it contains 61 residues (Table 2). It is the *N*-terminal 61-residue peptide of L chain K-25. The arginine-containing fragment A contains about 150 residues (not shown). Its *N*-terminal sequence, Phe-Lys-Gly-Ser, determined by the dansyl–Edman procedure, was identical with that of the homologous fragments A derived from L chains BS-1 and BS-5 (Jaton, 1974*a*,*b*). By homology with the known sequences of the V regions of L chains BS-1 and BS-5, citraconyl peptide A follows fragment B and extends therefore between positions 62 and 211.

Accordingly, fragments A_3 (residues 62–109) and A_1 (residues 110–211) were released from the large fragment A by mild acid cleavage of the Asp_{109}–Pro_{110} bond located between V and C domains of rabbit κ chains (Jaton, 1974*a*). The yields of fragments A, B, A_1 and A_3 were high (65–75%) and comparable with those described previously (Jaton, 1974*a*,*b*).

Sequence of fragment B (residues 1–61). The amino acid sequence of the *N*-terminal 41 residues was determined by subjecting the intact L chain (250nmol) to automated Edman degradation by using the Quadrol programme. A unique sequence was found; the repetitive yields of phenylthiohydantoin derivatives are represented in Fig. 2(*b*). The sequence of the *C*-terminal portion of fragment B was determined after isolation and characterization of the tryptic peptides T_4, T_5 and T_6 derived from the decitraconylated fragment B. Their amino acid com-

Fig. 4. *Comparison of V-region sequences of rabbit H chains of a_1, a_2 and a_3 allotypes*

The sequences of a_1 and a_3 normal pooled H chains are taken from Wilkinson (1969), Mole *et al.* (1971) and L. E. Mole (unpublished work); that of H chain R-2690 (allotype a_2) is from Fleischman (1971, 1973) and that of H chain BS-5 (allotype a_1) is from Jaton (1974*d*). Deletions (----) are shown to maximize the homology. In a_1 and a_3 pools, residues placed above or below the line at some positions in the sequences are alternative residues. Unidentified residues are indicated by a question mark in parentheses. Residues in boxes are probably allotype-related residues. The hypervariable sections are framed in broken lines, and the residues which are underlined are those which vary between the two anti-(type III pneumococci) antibodies BS-5 and K-25.

Table 2. *Amino acid composition of selected peptides from the V region of L chain K-25*

Values are residues/molecule of peptide. Values in parentheses are integral values confirmed by sequence analysis. Peptides T_4, T_5, T_6 and Ch-T_{4-2} are derived from fragment B; all others from fragment A_3. Mobilities are expressed relative to Asp (= −1.0) or to Arg (= +1.0). Yields of peptides are computed relative to the starting material (fragments B and A_3). N.D., not determined.

			Tryptic peptides			Thermolysin peptides				Chymotryptic peptides		
Amino acid	Fragment B	Fragment A_3	T_4	T_5	T_6	T-Th_{1a}	T-T_{1b}	Th_1	Th_2	Ch-T_{4-2}	Ch-Th_{2a}	Ch-Th_{2b}
Lys	4.4	2.2	—	1.1 (1)	1.0 (1)	—	1.1 (1)	1.0	—	—	—	—
Arg	1.0	—	1.0 (1)	—	—	—	—	—	—	1.0 (1)	—	—
CmCys	0.7	1.8	—	—	—	—	—	—	0.8	—	—	0.7 (1)
Asp	1.3	3.3	—	—	—	1.0 (1)	—	1.0	—	—	—	—
Thr	4.7	6.8	0.9 (1)	—	—	—	0.9 (1)	0.9	1.8	—	0.8 (1)	0.8 (1)
Ser	7.8	6.0	4.2 (4)	—	—	—	—	—	2.8	2.6 (3)	2.0 (2)	0.9 (1)
Glu	8.1	4.2	—	1.0 (1)	—	—	1.0 (1)	1.0	1.1	—	—	1.0 (1)
Pro	3.9	—	—	2.8 (3)	—	—	—	—	—	—	—	—
Gly	4.2	7.2	1.2 (1)	1.1 (1)	—	1.1 (1)	3.1 (3)	3.8	—	1.1 (1)	—	—
Ala	6.9	2.7	1.8 (2)	—	—	—	—	—	—	1.0 (1)	—	—
Val	4.5	2.4	1.0 (1)	—	—	—	2.8 (3)†	2.6†	—	0.9 (1)	—	
Met	—	—	—	—	—	—	—	—	—	—	—	—
Ile	3.0	0.8	—	—	0.9 (1)	—	—	—	—	—	—	—
Leu	5.1	2.1	1.0 (1)	—	2.1 (2)	—	—	—	—	—	—	—
Tyr	3.9	4.6	—	—	1.0 (1)	—	—	—	3.6	—	0.9 (1)	2.8 (3)
Phe	—	3.4	—	—	—	—	0.9 (1)	0.8	—	—	—	—
Trp	(1)*	—	—	—	—	—	—	—	—	—	—	—
Total residues	60.5	47.5	11	6	5	2	10	12	11	7	4	7
Mobility at pH6.5	N.D.	N.D.	+0.30	+0.36	+0.40	−0.80	0	−0.21	−0.46	+0.33	0	−0.66
Yield %			35	7	37	9	12	20	29		20	22

* Measured with Ehrlich reagent; found to be 1 residue/molecule of peptide.

† Value after 72h hydrolysis.

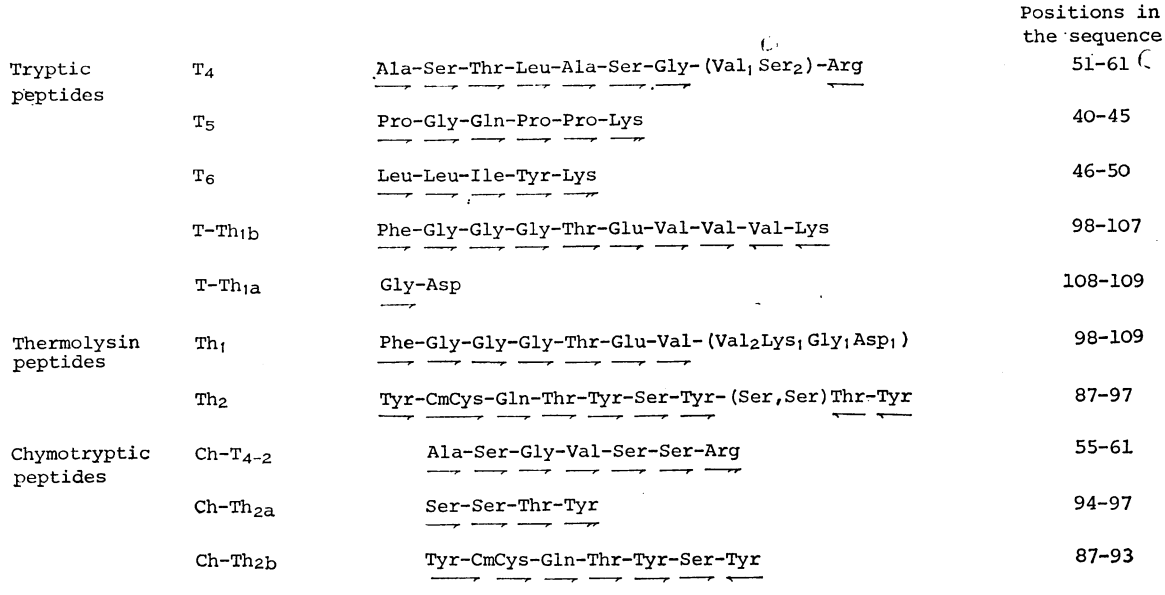

Fig. 5. *Sequences of selected peptides from the V region of L chain K-25*

Symbols are as in Fig. 3. The amino acid compositions and mobilities of the peptides are given in Table 2.

positions and sequences, which were determined by the dansyl–Edman procedure, are presented in Table 2 and Fig. 5. Peptide T_4 is the only arginine-containing peptide of the tryptic digest of fragment B; it is therefore the *C*-terminal peptide of fragment B. Peptide T_5 overlaps the sequence determined with the protein sequencer and peptide T_6 is placed by homology with L chains BS-1 and BS-5 between peptides T_5 and T_4 in positions 46–50 (Fig. 6).

Sequence of fragment A_3 (residues 62–109). The amino acid sequence between residues 62 and 90 was obtained by subjecting the large fragment A (residues 62–211, 500nmol) to automated Edman degradation. Fragment A was chosen rather than fragment A_3 because of less pronounced extraction of the peptide during the washing cycles. The yields of degradation are reported in Fig. 2(*b*). The *C*-terminal sequence of fragment A_3 (residues 91–109) was determined by conventional methods: a thermolysin digest of peptide A_3 yielded peptides Th_1 and Th_2. The amino acid composition of peptide Th_1 (Table 2) indicates that it is the *C*-terminal portion of fragment A_3 (Fig. 6). Peptide Th_2 overlaps the sequence of fragment A_3 determined with the sequencer and extends between positions 87 and 97. Peptide Th_2 was split by chymotrypsin into two peptides; their amino acid compositions and sequences are given in Table 2 and Fig. 5. The order of the peptides derived from fragment A_3 is as shown in Fig. 6.

The amino acid compositions of the two fragments B and A_3 of the V region (Table 2) agree very well with that calculated from the sequence, shown in Fig. 6.

N-Terminal sequence of fragment A_1 (C region residues 110–211). The *N*-terminal 36 residues of the C region of L chain K-25 were analysed in the protein sequencer. Details of the run are given in Fig. 2(*b*). The sequence is identical with that of the homologous fragments A_1 of L chains BS-1 and BS-5 (Fig. 6).

Discussion

The amino acid sequence of the V regions of both H and L chains derived from the anti-pneumococcal antibody K-25 was determined. Owing to the presence of two suitably placed methionine residues in the *N*-terminal half of the H chain and of only one arginine residue in the V region of the L chain, large fragments could be easily purified; their sequences were determined almost completely by the protein sequencer. The monoclonal character of antibody K-25 was confirmed by the finding of an unequivocal single sequence of the peptides. The alignment of several peptides of the V region of H chain K-25 was done by homology with the known V_H region sequences of the rabbit (Mole *et al.*, 1971; Fleischman, 1971, 1973; Jaton, 1974*d*). Only 3 μmol of antibody

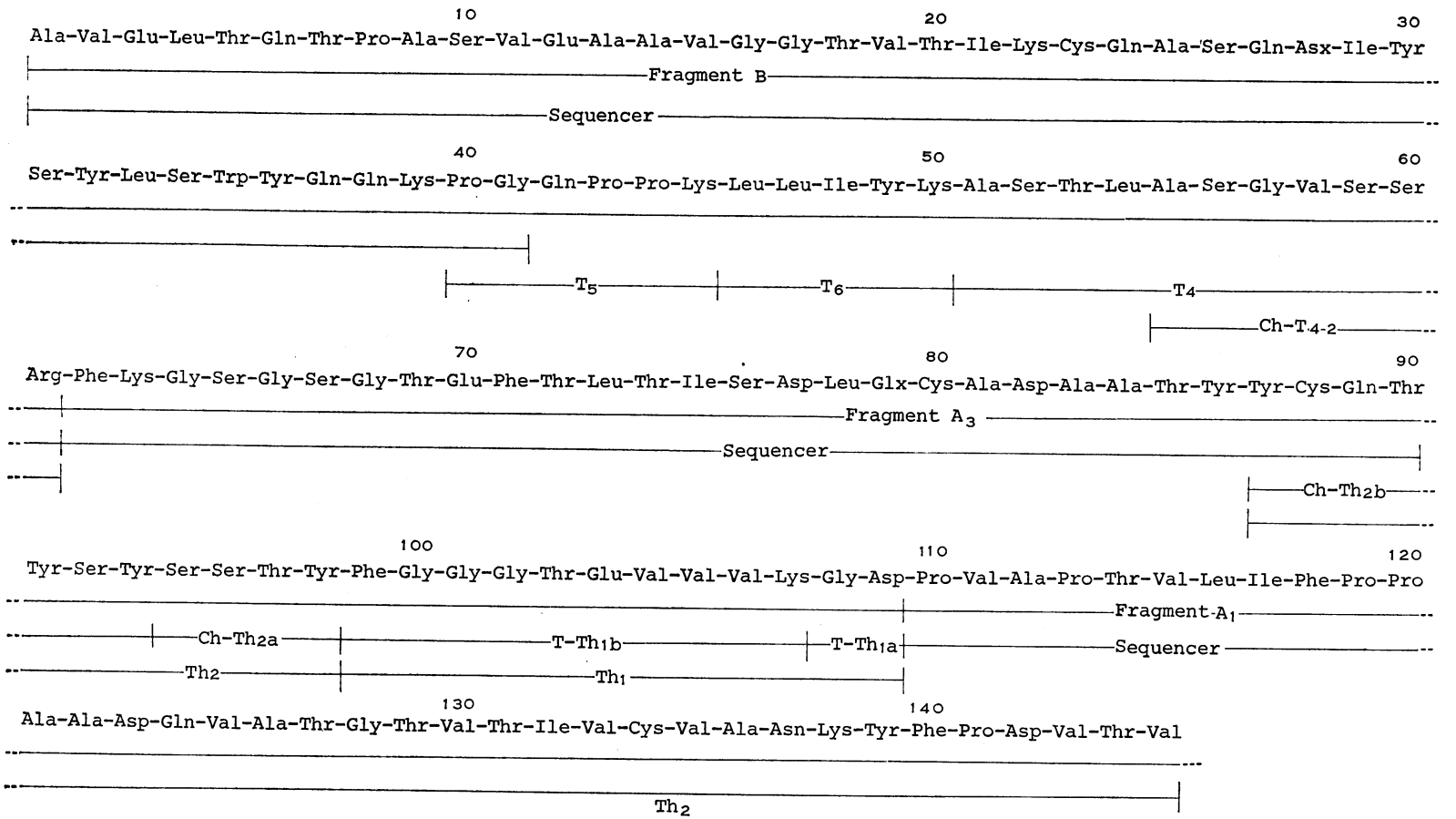

Fig. 6. *Amino acid sequence of the variable region of L chain K-25*

Residues 1–41, 62–90 and 110–145 were identified by the sequencer. T and Ch are tryptic and chymotryptic peptides respectively; see also Fig. 5 and Table 2 for details.

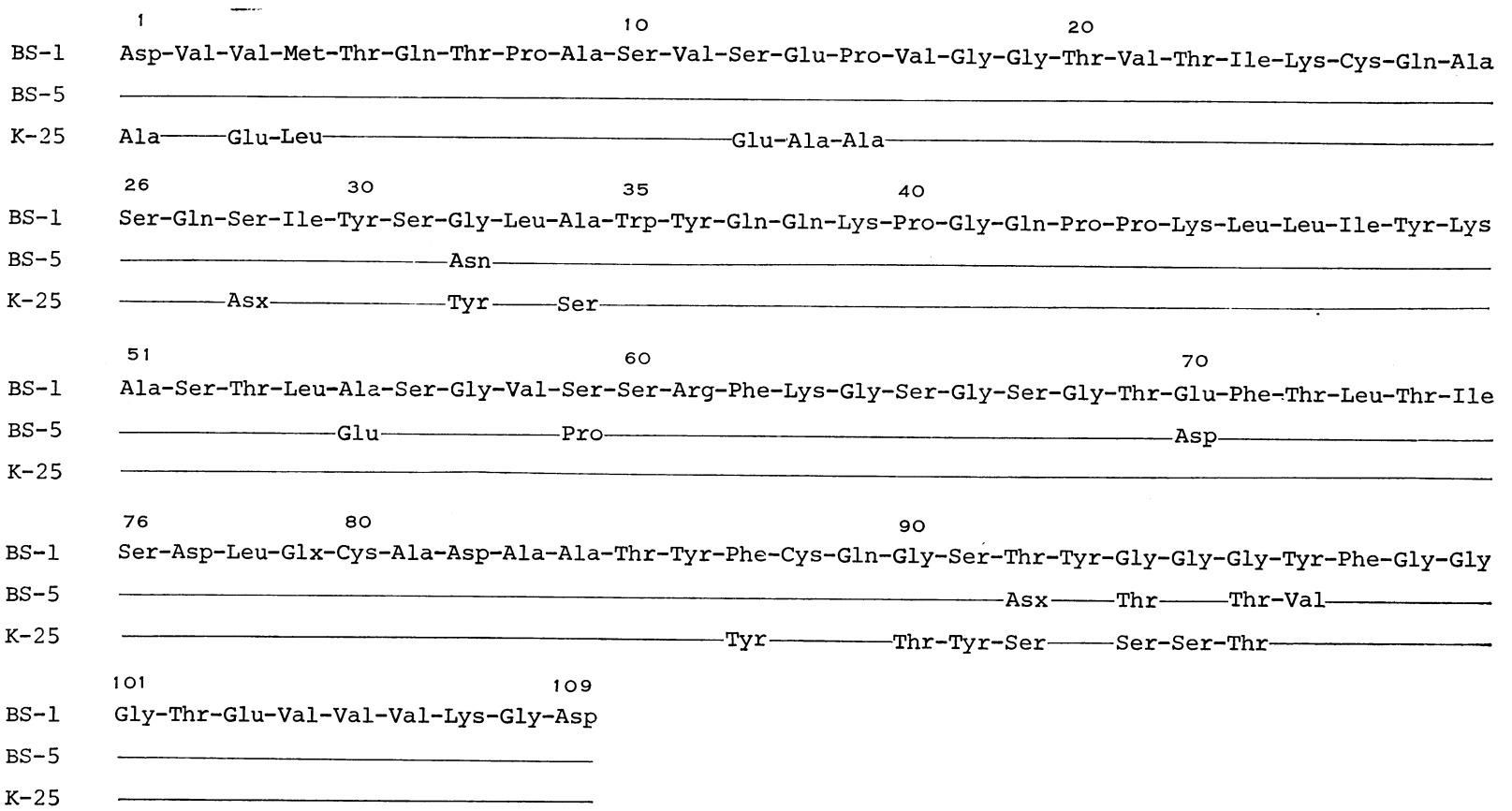

Fig. 7. *Comparison of the V_κ region sequences of rabbit L chains derived from antibodies to type III pneumococcal polysaccharide*
The solid line indicates sequence identical with that of L chain BS-1.

K-25 was needed to establish the complete sequence of the V domain.

When the V_H region of antibody K-25 is compared with the basic sequences of human V_H region subgroups (Capra & Kehoe, 1975), H chain K-25 exhibits only 27, 39 and 31% homology with V_{HI}, V_{HII} and V_{HIII} subgroups respectively. H chain K-25 thus appears more homologous to the V_{HII} subgroup than to the V_{HI} or V_{HIII} subgroup of variability.

The comparison of known V region sequences of a_1, a_2 and a_3 H chains is shown in Fig. 4. Outside the hypervariable sections, the sequence of H chain BS-5 (allotype a_1) agrees well with that of the a_1 pooled H chains (Mole *et al.*, 1971), except in positions 78–82. Evidence that amino acid-sequence variations in the latter section correlate with inheritable allelic variants specified by a locus different from locus *a* has been suggested (Porter, 1974). It is noteworthy that H chain BS-5 exhibits a lysine residue at position 78 and a proline residue at position 82. Two other homogeneous H chains of the same allotype a_1 as H chain BS-5 have a methionine and a threonine residue respectively at those homologous positions (Jaton *et al.*, 1973). The significance of these findings in genetic terms is as yet unclear.

The two a_2 chains K-25 and R-2690 (Fleischman, 1971, 1973) differ from each other by one interchange within the first 70 residues only, at position 42 (hypervariable sections not counted).

On the basis of all available data at the present time, allotype-related differences between a_1, a_2 and a_3 H chain V regions appear to occur within the *N*-terminal 16 residues. However, it cannot be ruled out that other positions, such as 64, 69–70 and 83–84, may also be allotype-related.

The hypothesis that each rabbit would have all structural genes coding for a_1, a_2 and a_3 allotypes under the control of regulator genes has been put forward (see, e.g. Fudenberg *et al.*, 1972). Evidence for the presence of all three allotypic markers of the group *a* and of three of the four markers of the group *b* in the serum of a single hyperimmunized rabbit has been documented (Strosberg *et al.*, 1974). If genetic studies substantiate this observation, then it is reasonable to assume that the allotypes are not truly allelic for structural genes (Kelus & Gell, 1967) but rather for regulator genes. A similar hypothesis has been proposed for the control of the synthesis of b_4 and b_5 allotypes in L chains (Bell & Dray, 1971).

H chains of antibodies K-25 and BS-5 differ by two residues in the first hypervariable section at positions 30 and 34, by two residues in the second hypervariable section at positions 49 and 60, and markedly in the third hypervariable zone by seven amino acid interchanges. In the latter section, it is nonetheless remarkable that antibodies BS-5 and K-25 exhibit four identical residues at positions 96 (Thr), 97 (Gly), 98 (Leu) and 101 (Leu). The present data do not allow one to decide whether these residues are characteristic structural features of the anti-pneumococcal type III specificity or whether they are constant residues common to the binding sites of some rabbit antibodies. There is an insertion of one serine residue in antibody K-25, relative to antibody BS-5 (position 103, Fig. 4). Antibody K-17, also directed against type III pneumococci, exhibits an insertion of four residues when compared with antibody BS-5 (Strosberg *et al.*, 1972). The length of this section is therefore variable, whereas that of the homologous section of the corresponding L chains appears constant (Fig. 7). This suggests that the shape of the binding site is variable, thus giving rise to molecules with differing affinities for the polysaccharide ligand. Noteworthy is the presence of one lysine residue (at position 102) in antibody K-25 which may be involved in ionic linkage with the cellobiuronic acid determinant of the antigen in the binding site (Speyer *et al.*, 1973). The existence of basic residues in the site is, however, not a prerequisite, as the site of antibody BS-5 is devoid of basic residues (Jaton, 1974*d*). Direct assessment of the residues in contact with the antigen will be best made by the examination of the crystallographic model of the antibody.

The V region of L chain K-25 is compared with that of L chains BS-1 and BS-5 in Fig. 7. L chain K-25 differs from L chain BS-5 by 19 amino acids, of which 11 are found in the three hypervariable sections (Kabat & Wu, 1971). When comparison is made with L chain BS-1, 16 substitutions are found; in view of the seven amino acid interchanges within the *N*-terminal 28 residues, L chain K-25 belongs to a different subgroup of rabbit κ chains (Braun & Jaton, 1973), although rabbit subgroups are less easily distinguished than human subgroups when the whole V region is considered. Similarly to their counterpart H chains, these three V_L regions are very similar within the first and second hypervariable sections but differ markedly in the third zone, i.e. between residues 87 and 97. This suggests that the first and second hypervariable sections of both chains could control the 'coarse' specificity of the pneumococcal antibody, whereas the third hypervariable sections could determine the 'fine' specificity.

Recombination of chains from these antibodies indicates that a fully active binding site is regenerated only when autologous H and L chains are recombined; pairing of a H chain with a heterologous L chain exhibiting six amino acid substitutions in the hypervariable sections leads to an immunoglobulin which has little, if any, of the activity of the autologous recombinant molecule. This indicates that the complementary interactions between the homologous hypervariable sections of the V domain are likely to dictate the specificity of the immunoglobulin molecule (Huser *et al.*, 1975).

I thank Dr. E. M. Press for helpful comments and criticisms of the manuscript and Mr. W. Hunkeler for his excellent technical assistance.

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