THE AMINO ACID SEQUENCE OF THE LIGHT-CHAIN VARIABLE REGION OF A RABBIT ANTIBODY AGAINST THE STREPTOCOCCAL GROUP A VARIANT POLYSACCHARIDE (ANTIBODY K16–167)

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1. Introduction

Comparative studies of the amino acid sequences of myeloma proteins have contributed significantly to the knowledge of the structure of antibody molecules and have revealed an apparently limitless variability of the immunoglobulins. The mechanism by which this variability is generated is still an unresolved problem. Amino acid sequence analysis of homogeneous antibodies induced by antigens of great epitope homogeneity and derived from animals of defined genetic background are expected to provide insights into the generation of antibody diversity. Rabbits selectively bred for oligoclonal responses against the streptococcal group A variant polysaccharide (Av-CHO) have proved a particularly fruitful system for the analysis of amino acid sequence patterns [1–4].

A previous report from this laboratory has compared the amino acid sequences of several variable halves of light-chains derived from anti-Av-CHO antibodies [4]. We would like to present in detail the amino acid sequence of the variable region of the light-chain (V_L) of the rabbit antibody K16-167, another anti-Av-CHO antibody of the b4 allotype.

2. Materials and methods

Rabbit K16-167 was immunized with streptococcal group A variant (strain A486 variant, M-) vaccine as described [5]. The antibody was isolated from the hyperimmune serum by repeated preparative agarose block electrophoresis [6].

Heavy- and light-chains were prepared by partial reduction and alkylation, followed by separation on Sephadex G-100 in 1 M acetic acid [7]. Complete reduction and alkylation with iodo-[2-^{14}C]acetic acid was done according to O'Donnell et al. [8]. Succinylation of fully reduced and alkylated light-chain was carried out as outlined by Klotz [9]. Citraconylation was done by the method of Gibbons and Perham [10]. The unblocking of citraconyl-peptides was effected by incubation in acetate buffer, pH 4.2, for 6 h at 37°C [11].

Tryptic digestion was performed for 4 h by using a 1:100 ratio (w/w) of TPCK-trypsin to protein in the presence of 1% NH_4HCO_3. Hydrolyses with carboxypeptidases A and B were conducted as recommended by Ambler [12].

Preparative high-voltage paper electrophoresis was done according to Press et al. [13]. Peptides were detected as described elsewhere [14,15]. They were eluted from the paper with 0.02 M NH_4OH.

Automated sequence determination of the N-terminal section of the light-chain and of large peptides was performed as previously described [16]. Small peptides were sequenced automatically after attachment of 2-amino-1,5 naphthalene disulfonic acid to the C-terminal residue [17]. Phenylthiohydantoin (PTH) derivatives of amino acids were analyzed by gas chromatography [18], thin-layer chromatography on silica plates [19] and by amino acid analysis after conversion of the PTH derivatives into free amino acids by hydrolysis with HI [20]. Halfcystine residues were identified as *S*-[^{14}C]carboxymethyl derivatives by liquid scintillation counting.

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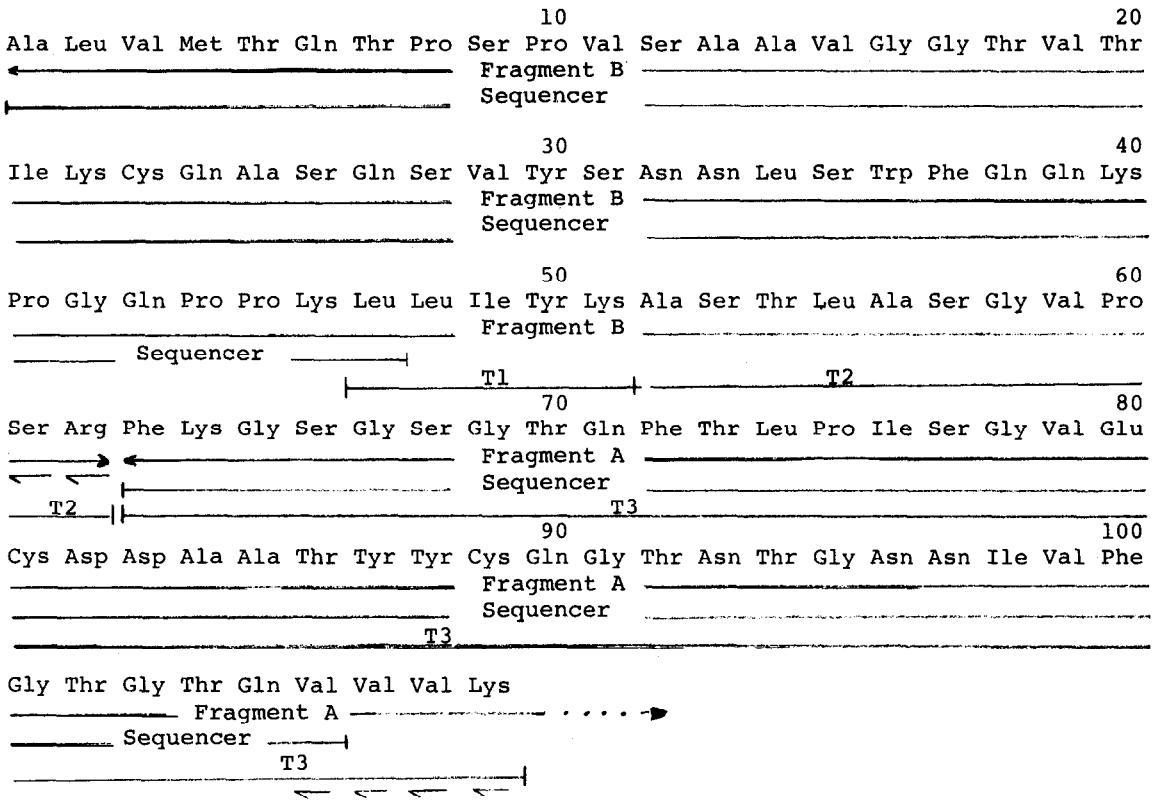

Fig.1. Amino acid sequence of V_L K16-167. The indicated large peptides (A and B) were isolated from a Bio-Gel $A_{1.5}$ m column in 5 M guanidine hydrochloride after tryptic digestion of the citraconylated light chain. Tryptic peptides T_1 and T_2 were obtained from fragment B after decitraconylation and isolated by high-voltage paper electrophoresis.

3. Results

The amino acid sequence of the V_L region of rabbit antibody 16-167 is displayed in fig.1. The N-terminal 47 amino acid residues were established by automated sequencing of the intact light-chain. The repetitive yield in this sequenator run was 94%. Amino acid analysis of the entire light-chain (not shown) indicated the presence of two arginine residues. Advantage was taken of this fact by succinylation and tryptic digesiton of the light-chain followed by dialysis against distilled water, lyophilization and automated sequencing of the mixture. The finding of a single amino acid sequence confirmed the presence of only one arginine residue in the V_L region indicating that the second arginine (presumably in position 207) was from the constant region [21]. Under these

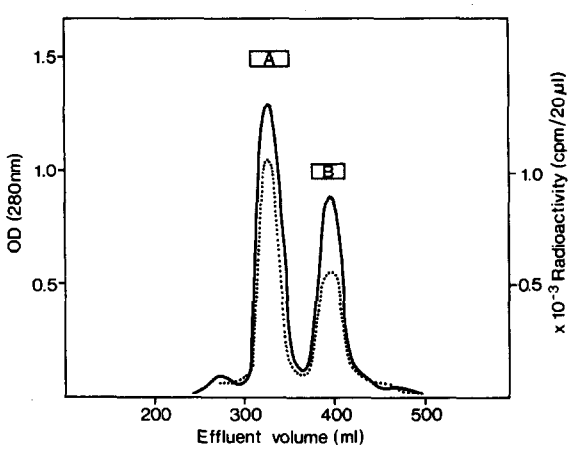

Fig.2. Elution-profile of the tryptic digest of the citraconylated V_L K16-167 on a Bio-Gel $A_{1.5}$ m column (2 × 180 cm). Fragments A and B are indicated.

conditions the C-terminal tripeptide Gly–Asp–Cys was presumably dialysed out. It could be shown by homology with other rabbit κ-chains [21–23] that the unblocked peptide of this mixture (peptide A in fig.2) starts with phenylalanine in position 63 and comprises the third hypervariable region and the constant part of the chain. Peptide A and peptide B, which comprises the 62 N-terminal residues of the light-chain, moreover were obtained by tryptic digestion of the citraconylated light-chain and separation on Bio-Gel $A_{1.5\,m}$ in 5 M guanidine hydrochloride (fig.2). After decitraconylation and tryptic digestion of fragment B, two relevant peptides (T_1 and T_2) were isolated by high-voltage paper electrophoresis. Their amino acid sequences were established by automated Edman degradation, as described in Materials and methods, and by hydrolysis with carboxypeptidases A and B (fig.1).

Peptide T_3 starting with phenylalanine in position 63 and comprising the third hypervariable region up to lysine in position 107 was isolated, after decitraconylation and tryptic digestion of peptide B, by two cycles of gel-filtration on Sephadex G-50 in 5 M guanidine hydrochloride. The C-terminal sequence of this peptide was established by time-dependent digestion with carboxypeptidases A and B (fig.1).

4. Discussion

The primary structure of the V_L of rabbit antibody K16-167 was established by sequencing 4 different peptides, i.e., the N-terminus of the intact chain, the N-terminus of a large peptide starting with phenylalanine in position 63 and ending with the C-terminal arginyl residue (position 207) of this chain, and two

Table 1
Degree (%) of homology of rabbit and human V_L regions

Framework positions			Hypervariable positions									
			Anti-Av-CHO			Anti-SIII			V_κI	V_κII	V_κIII	V_κIV
			K16-167	K4820	K9-335	BS-1	BS-5	K-25	Roy	Tew	Ti	Len
Anti-Av-CHO		K16-167	–	71	79	62	67	54	29	31	17	29
		K4820	81	–	67	58	62	54	42	29	46	29
		K9-335	84	80	–	58	67	58	29	25	33	33
Anti-SIII		BS-1	80	86	82	–	75	71	37	21	37	29
		BS-5	80	85	82	98	–	67	50	21	37	42
		K-25	81	86	77	88	86	–	42	25	50	33
V_κI		Roy	64	70	59	70	70	69	–	10	43	18
V_κII		Tew	56	57	57	56	57	53	61	–	24	32
V_κIII		Ti	60	57	59	62	62	57	60	72	–	32
V_κIV		Len	60	63	66	66	66	63	73	77	76	–

Hypervariable positions, numbering scheme according to Schneider and Hilschmann [24]. Positions were aligned to maximal homology. Deletions were taken as identical positions.

small tryptic peptides, one overlapping with the N-terminal sequence. Since rabbit anti-Av-CHO antibodies do not display a second hypervariable region [4] no difficulties occurred with the positioning of the peptides by homology with other rabbit light-chain sequences [4,21–23].

The degrees of homology of V_L of antibody K16-167 to human light-chains of subgroups I–IV [25] and to V_L of rabbit antibodies are shown in table 1. Within invariant positions (framework) rabbit light-chains share between 77–98% homology, whereas human light-chains of different subgroups share only 61–77% homology; human and rabbit light-chains share between 53% and 70% homology. The analysis of similarities within the 3 hypervariable or complementarity-determining regions [25] indicates 10–43% homology between human light-chain hypervariable regions and 17–50% homology between human and rabbit hypervariable regions. Hypervariable regions of rabbit light-chains derived from anti-Av-CHO antibodies, however, share between 67% and 79% homology (the sequences of light chains K4820 and K9-335 are taken from ref. [4]). This high extent of homology is partly due to the number of deletions in the first hypervariable section which were taken as identical positions. In view of the possible importance of the chain-length for the formation of the combining site [26] this assumption seems to be justified. Moreover, it has to be considered that rabbit light-chains lack a second hypervariable region [4]. If the comparison is therefore restricted to positions of the first and third hypervariable regions, still 53%, 59% and 71% homology are observed between the three light-chains K4820, K16-167 and K9-335 derived from anti-Av-CHO antibodies.

The comparison of light-chain hypervariable regions of rabbit anti-Av-CHO and anti-Type III pneumococcal polysaccharide (SIII) antibodies, indicates between 54% and 67% homology, whereas among hypervariable regions of anti-SIII antibodies 67–75% homology is noted, which is similar to the homology observed among anti-Av-CHO antibodies. It thus appears that light-chains derived from rabbit antibodies with the same binding activity show a slightly higher degree of homology within their hypervariable regions than rabbit antibodies with different binding activities. In addition a higher extent of homology is noted among hypervariable regions of different light-chains derived from rabbit anti-polysaccharide antibodies than among hypervariable regions of human light-chains or across the two species. The variability of anti-polysaccharide rabbit antibody V_L regions is therefore less than that of randomly chosen immunoglobulin light-chains but more than that of heavy-chain variable regions of anti-phosphoryl-choline antibodies (for reference see [27]).

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