AMINO-TERMINAL SEQUENCES OF BLOCKED κ-CHAINS FROM HOMOGENEOUS RABBIT ANTIBODIES

Alan P. JOHNSTONE and Thomas J. KINDT
The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA

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1. Introduction

Amino acid sequence determinations of immunoglobulin chains have given considerable insight into the genetics of antibody diversity (for review, see ref. [1]). Primary sequence studies on rabbit immunoglobulin chains are facilitated by the use of hyperimmunization of rabbits with bacterial vaccines to elicit the production of high concentrations of molecularly uniform antibodies [2]. The majority (approximately 90%) of the rabbit immunoglobulin light chain pool is of the κ-type, having an unblocked N-terminus; considerable sequence data on these unblocked chains have been derived from structural studies of homogeneous antibodies [3,4]. However, several κ-chains having blocked N-termini have recently been reported [5,6]; little structural data are available on these blocked chains.

In this report, the amino-terminal sequences of six such homogeneous blocked κ-chains are presented. These sequences are homologous to unblocked κ-chains, indicating that the blocked chains are derived from the major unblocked population by post-translational processing and are not encoded by different genes.

2. Materials and methods

2.1. *Enzymes*

Pronase was purchased from Sigma Chemical Co., St. Louis, Mo., and carboxypeptidase A from Worthington Biochemical Co., Freehold, NJ.

2.2. *Blocked κ-chains*

Light chains 3322-D [6] and 3392 were a gift of Drs L. E. Cannon and M. N. Margolies. Other light chains were isolated [7] in this laboratory following partial reduction and alkylation of homogeneous antibodies elicited by hyperimmunization of rabbits with streptococcal vaccines [2]. These κ-chains were classified as having blocked N-termini by the absence of detectable levels of phenylthiohydantoin-amino acids upon automated sequence analysis [5,6].

2.3. *Pronase digestion of light-chains*

The blocked κ-chains (2.5 mg) were completely reduced in 7 M guanidine–HCl, 0.5 M Tris–HCl, pH 8.2 (10 mg/ml) using 5 mM dithioerythritol for 2 h, and alkylated with 10.5 mM iodoacetamide for 30 min, all at room temperature. Excess reagents were removed by dialysis against 0.1 M NH_4OH and the proteins lyophilized.

Pronase digestion (enzyme to substrate ratio, 1:25, w/w) was carried out in 1% NH_4HCO_3 buffer, pH 8.2 (5 mg/ml) for 3 h at 37°C, followed by lyophilization to terminate the digestion and remove the buffer. The digest was then applied to a column (1.3 × 1.5 cm, 2 ml) of Amberlite IR-120 (H^+-form), which had been exhaustively washed with water. Peptides containing no free amino group were purified from the unretarded fraction by paper electrophoresis [8].

2.4. *Cyanogen bromide cleavage of 3664 κ-chain*

The κ-chain (10 mg) was cleaved with 50 mg CNBr in 0.5 ml 70% (v/v) formic acid for 24 h at 4°C. The mixture was then applied directly to a column (1.2 × 30 cm) of Sephadex G-25 medium in 50% (v/v) formic acid, and the blocked N-terminal peptide was purified by paper electrophoresis from the included fractions.

2.5. *Analytical methods*

Amino acid analysis was performed using a Durrum D-500 analyzer following hydrolysis in constant boiling HCl, in vacuo, for 72 h (3664 peptides) or 22 h at 110°C. The dried hydrolysates of peptides containing homoserine were dissolved in 0.5 ml pyridine–acetate buffer, pH 6.5, and heated at 100°C for 1 h before analysis [9].

High voltage paper electrophoresis was carried out using the apparatus and buffers described previously [10]. The N-terminal peptides, having no free amino group, were identified as such by their positive reaction with the hypochlorite–starch–iodide reagent [11] and their lack of reaction with ninhydrin. The mobility of all peptides was measured relative to pyrrolid-2-one-5-carboxylic acid (−1.0) and ε-Dnp–lysine (0).

Digestion with carboxypeptidase A (0.5 μg enzyme/nmol peptide) was carried out in 0.5% NH_4HCO_3 buffer, pH 8.4, (0.2 nmol/μl) at 37°C for periods from 0.5–24 h; the amino acid(s) released was identified on the Durrum D-500 analyzer.

2.6. *Quantitation of* b-*locus markers on κ-chains*

Quantitative determination of *b*-allotypes was carried out using insolubilized anti-*b*-allotype anti-sera in a radioimmunoassay as described previously [12].

3. Results and discussion

More than 80% of each blocked light chain preparation was bound by insolubilized antisera specific for the *b*-locus (κ chain constant region) allotypes (table 1). This established that the blocked chains were of the κ- rather than λ-type.

Following pronase digestion, a single blocked peptide was isolated in good yield from each κ-chain (table 1), indicating that the chains were homogeneous in their N-terminal region, although the lower yield from some chains (e.g. 3392) might reflect the presence of a minor undetected variant. The pronase peptides were characterized from their amino acid composition and their mobility on paper electrophoresis [13]. In addition, the tripeptide from 3664 was digested with carboxypeptidase A, which released valine 0.9 mol/mol peptide after 0.5 h. Each blocked pronase peptide contained glutamic acid on analysis and pyrrolid-2-one-5-carboxylic acid was assumed to be the N-terminal amino acid in each case. The proposed structure of these peptides is presented in table 1; the peptide □Glu–Val was isolated from five of the six chains studied.

The N-terminal sequence of two κ-chains (4035 and 3664) was investigated further. Pronase digestion

Table 1
N-Terminal pronase peptides of blocked κ-chains

κ-Chain designation	Vaccine used to elicit antibody[a]	κ-Chain allotype	Isolated blocked pronase peptides				
			Amino acid composition[b]	Mobility		Yield[c]	Proposed sequence
				pH 6.5	pH 3.5		
3664	Strep C	b4	$Glu_{1.16}$,$Val_{1.00}$,$Ile_{0.93}$	−0.51	−0.35	0.65	□Glu–Ile–Val
3660	Strep A	b4	$Glu_{1.10}$,$Val_{1.00}$	−0.69	−0.55	0.60	□Glu–Val
4035	Strep C	b4	$Glu_{1.16}$,$Val_{1.00}$	−0.66	−0.55	0.55	□Glu–Val
4295-7	Strep C	b5	$Glu_{1.16}$,$Val_{1.00}$	−0.67		0.68	□Glu–Val
3322-D	Pneu VIII	b5	$Glu_{1.15}$,$Val_{1.00}$	−0.67		0.59	□Glu–Val
3392	Pneu III	b4	$Glu_{1.18}$,$Val_{1.00}$	−0.66		0.47	□Glu–Val

[a] Strep C, Strep A, Pneu VIII, Pneu III designate the streptococcal Group C, Group A, pneumococcal type VIII, type III vaccines, respectively

[b] Amino acid compositons were determined following 72 h hydrolysis of the 3664 peptide and 22 h hydrolysis of all other peptides

[c] Yields are expressed as mol peptide/mol κ-chain after ion-exchange chromatography

of 4035 under less harsh conditions (enzyme to substrate ratio of 1:70, by weight, for 3 h at 37°C) released ⌐Glu–Val in lower yield than above, and a second blocked peptide (composition: $Glu_{1.26}$, $Thr_{1.20}$, $Val_{1.00}$, $Leu_{0.81}$) was isolated in a yield of 0.22 mol/mol of κ-chain. The mobility of this second peptide at pH 6.5 (–0.45) indicated a charge of -1 for a tetrapeptide; the C-terminal sequence was determined as –Leu–Thr by digestion with carboxypeptidase A. The structure ⌐Glu–Val–Leu–Thr– is therefore proposed for the N-terminus of 4035.

Only one light chain of the six (3664) contained methionine (0.89 mol/mol κ-chain) upon analysis. By homology with unblocked κ-chains, the methionine residue was assumed to be near the N-terminus, and cleavage with CNBr released an N-terminal peptide with the composition: $Glu_{1.00}$, $Val_{1.00}$, $Ile_{0.82}$, $Hse_{0.92}$. The peptide was recovered in low yield (0.13 mol/mol κ-chain after gel filtration), presumably because of the resistance of the –Met–Thr– sequence in light chains to CNBr cleavage [14]. The mobility of this peptide at pH 6.5 (–0.40) indicated a charge of –1 for a tetrapeptide; digestion with carboxypeptidase A allowed the C-terminal sequence to be determined as –Val–Hse. The proposed structure of this peptide is ⌐Glu–Ile–Val–Hse.

Two different N-terminal sequences were therefore found for the blocked κ-chains (fig.1); both of these differ from the sequence of blocked λ-chains [15]. The variation in sequence did not correlate with the constant region allotype, the vaccine used to elicit the antibody (table 1) or the pedigree of the rabbit producing the light chain (rabbits 4035 and 3664 had the same parents, and rabbits 3322 and 3392 belonged to a different colony from the others).

The blocked sequences are homologous to two common sequences of unblocked κ-chain (fig.1). The –Ile–Val– sequence (residues 2 and 3) in blocked chains correlates with the presence of methionine at position 4, as found in unblocked chains [4]. Internal glutamine or glutamic acid residues are present in some unblocked molecules [4] at position 1 of the sequence group 1 and position 2 of the sequence group (see fig.1). It is proposed that the pyrrolid-2-one-5-carboxylic acid residue of blocked κ-chains arises during post-translational processing of the precursor molecule, by non-enzymatic cyclization of an N-terminal glutamine residue, as suggested for mouse λ-chain [16]. The blocked κ-chains are therefore derived from the same source as unblocked κ-chains, and are probably not encoded by different genes.

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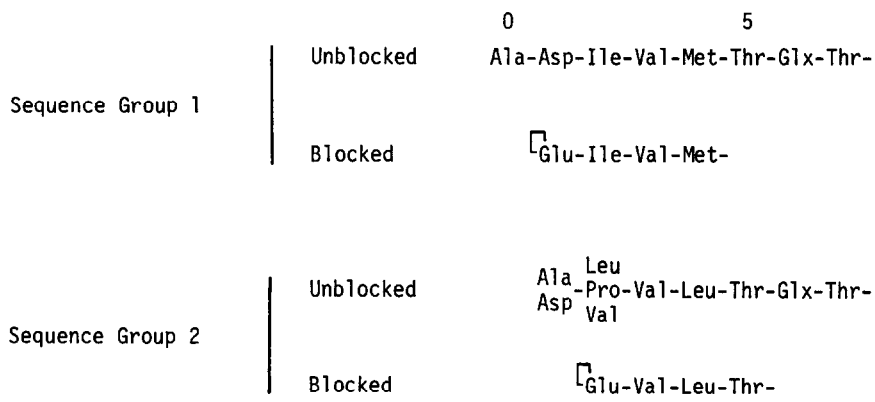

Fig.1. N-Terminal sequences of blocked and unblocked κ-chains. Blocked κ-chains 3664 and 4035 are compared with two common sequences of unblocked κ-chains (from ref. [4]). The numbering system is from ref. [4].

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