Intrachain Disulphide Bridges of Rabbit Immunoglobulin Light Chains of Allotypes b4 and b5

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The study of rabbit H-chain allotypes has provided important insights into the genetic control of immunoglobulin synthesis. For example, the V regions of the H-chains, which are governed by the a locus, are not class (C region)-specific (Todd, 1963; Wilkinson, 1969), and genetic markers in the C region of the γ -chain are closely linked to the a locus (Landucci Tosi *et al.*, 1970; Kindt *et al.*, 1970). As far as rabbit L-chains are concerned, the amino acid sequences at the C-termini are known to be related to allotypes controlled by the b locus (Appella *et al.*, 1969; Frangione, 1969), and we and others have been

attempting to find additional allotype-related sequences in rabbit κ -chains (Frangione & Lamm, 1970; Hood *et al.*, 1971; Chersi *et al.*, 1971). Aside from correlations with allotype, the primary structure and folding of rabbit L-chains are of interest from other viewpoints in addition to their obvious relationship to antibody specificity. Thus the intrachain-disulphide-bridge regions of immunoglobulins appear to have been conserved during evolution and are thought to be important determinants of folding (Milstein *et al.*, 1967; Edelman & Gall, 1969; Poljack *et al.*, 1972). In this regard, however, rabbit

Table 1. Peptides isolated from rabbit L-chains of allotypes b4 and b5

Peptides containing small letters were isolated after radioactive alkylation (Frangione & Lamm, 1970), and peptides containing capital letters were isolated by diagonal electrophoresis. Mobilities at pH 6.5 are expressed relative to aspartic acid as 1 and ϵ -Dnp-lysine as 0. The numbered positions of the cysteine residues are based on homologies with human and mouse κ -chains (Putnam *et al.*, 1967; Dreyer *et al.*, 1967).

Suggested position of cysteine residue	Peptide	Allotype	Mobility	Sequence
23	2b	b4	0.50	Ile-Asn-Cys
23	2b	b5	0.50	Ile-Asn-Cys
88	1a1	b4	0.30	Tyr-Tyr-Cys-Gln-Gln-Gly-Ser-Tyr
88	1a2	b4	0.37	Tyr-Tyr-Cys-Gln-Gly-Ser Ala
88	1b1a	b4	0.33	Tyr-Tyr-Cys-Gln-Gln-Ser-Asn Gly
88	1b1	b5	0.30	Tyr-Tyr(Cys,Gln,Gln,Gly)Ser-Tyr
88	1b1'	b5	0.35	Tyr-Tyr(Cys,Gln,Gln,Gly,Ser,Asp _{0.5} ,Ala _{0.5})
V region	3b	b4	1.00	Glu-Cys(Asp,Asp,Ala,Ala)
V region	4c, 2C	b4	0.93	Glu-Cys(Ala,Asp,Ala,Ala)Thr
V region	2D	b4	0.85	Leu Val-Glu-Cys-Asx(Asp,Ala,Ala,Thr) Ala
V region	5b, 5a	b5	0.95	Glu-Cys-Asx-Asx(Ala,Ala,Thr)
V region	4b, 2C	b5	0.93	Glu-Cys-Ala-Asp-Ala(Ala,Thr)
V region	2D	b5	0.85	Leu Val-Glu-Cys(Asx,Asp,Ala,Ala,Thr) Ala
171	1b3a	b4	0.45	Thr-Pro-Glx-Asx-Ser-Ala-Asx(Cys,Thr)
171	2E	b4	0.41	Thr-Pro-Glx-Asx(Ser,Ala,Asx,Cys,Thr)(Tyr,Asn,Leu)
171	2a	b5	0.75	Thr-Pro-Gln-Asn-Ser-Asp-Asp-Cys-Thr
171	2E	b5	0.61	Thr-Pro-Gln-Asn(Ser,Asp,Asp,Cys,Thr)(Tyr,Asn,Leu)
134	5A	b4	0	Ile-Val-Cys(Val,Ala,Asn)Lys
134	5A	b5	0	Ile-Val-Cys-Val(Ala,Asn)Lys
194	5B	b4	0.40	Glu-Tyr-Thr-Cys-Lys
194	5B	b5	0.40	Glu(Tyr,Thr,Cys)Lys

Fig. 1. Diagonal peptide 'maps' at pH 3.5 of normal b4 (a) and b5 (b) L-chains

●, Electrophoretic markers. Vertical column 2 contains the cysteine 'V region'—cysteine-171 bridge. The difference in location of peptide 2E in the two 'maps' is due to the alanine/aspartic acid interchange at position 169. Column 4 contains the cysteine-23- and cysteine-88-containing peptides, and column 5 the cysteine-134- and cysteine-194-containing peptides.

κ -chains are unusual in that most contain three intrachain cystine bridges (Rejnek *et al.*, 1969) instead of the usual two. Therefore we have also been interested in the pairing of the cysteine residues of the rabbit κ -chain.

In a previous communication (Frangione & Lamm, 1970) we reported the amino acid sequences of a number of peptides obtained from the normal L-chains of b4 and b5 rabbits after complete reduction and alkylation, an approach that gives no information on the pairing of the cysteine residues. We now report results obtained by diagonal electrophoresis of normal b4 and b5 L-chains and offer a proposal for the arrangement of the intrachain disulphide bridges.

L-chains were isolated from partially reduced and alkylated immunoglobulin G (Prahl & Porter, 1968) obtained from a1,b4 (gift from Dr. A. S. Kelus) and a3,b5 (The Jackson Laboratory) rabbits. Diagonal peptide 'maps' (Brown & Hartley, 1966) of peptic-tryptic digests of b4 and b5 L-chains were made at pH 3.5. The cysteine acid-containing peptides were isolated accordingly, and were purified further by electrophoresis at pH 6.5 and in some cases at pH 1.9 as well. Amino acid sequences were determined by the dansyl-Edman (Gray, 1967a,b; Frangione & Milstein, 1968) and/or subtractive Edman techniques. Side chains of Asx and Glx residues were assigned according to the electrophoretic mobility of peptides at pH 6.5 (Offord, 1966). In

Table 1 the sequences of the cysteine-containing peptides from the b4 and b5 L-chains are tabulated. The abbreviations used for peptides isolated by diagonal electrophoresis contain capital letters, and their positions on the diagonal 'maps' are designated as follows. Numbers 2, 4 and 5 refer to vertical columns in Fig. 1, column 2 containing the most acidic and column 5 the most basic peptides in the first dimension. The capital letters refer to cysteine acid-containing peptides in the second (post-oxidation) dimension. The abbreviations for peptides isolated after complete reduction and [^{14}C]carboxymethylation (Frangione & Lamm, 1970) contain small letters.

The peptides listed as containing cysteine-134 and cysteine-194 [human κ -chain numbering (Putnam *et al.*, 1967)] were located in column 5 of the diagonal 'maps' of b4 and b5 L-chains, consistent with their being paired in the native L-chain. Their ready isolation suggests an origin from the C region [see also Appella *et al.* (1971), who obtained these peptides in good yield from b4 L-chains].

The peptides listed as containing cysteine-23 and cysteine-88 were isolated and their sequences determined only after reduction and radioactive alkylation. This family of peptides lies in column 4 of the diagonal 'maps' because low-yield peptides consistent with peptides 1a, 1b and 2b were found there in contaminated form. The fact that we could isolate these peptides in better yields only after reduction and

radioactive alkylation (a more sensitive method than diagonal electrophoresis) and the occurrence of several variants of the Tyr-Tyr-Cys-containing peptide (Frangione & Lamm, 1970) suggest that they come from the V region. By homology with other κ -chains, the cysteine residue of the Tyr-Tyr-Cys sequence can be placed at cysteine-88. [The Tyr-Tyr-Cys triplet also occurs in this region in human λ -chains (Putnam *et al.*, 1967; Milstein *et al.*, 1968)]. Following this particular cysteine residue is a highly variable stretch (Frangione & Lamm, 1970) that is probably analogous to the hypervariable segment (positions 95–115) of the rabbit γ -chain (Mole *et al.*, 1971). Appella *et al.* (1971) have evidence that the cysteine residue in peptide 2b corresponds to position 23. Thus the cysteine residues designated as 23 and 88 in Table 1 form a cystine bridge in the V region of the native L-chain.

Two cysteic acid-containing peptides were found in column 2 (Fig. 1), and we believe they form the third, i.e. the 'extra' (Rejnek *et al.*, 1969), intrachain disulphide bridge of the rabbit κ -chain. By homology the cysteine residue of one of these can be tentatively placed at residue position 171, where serine occurs in human and mouse κ -chains. Studies on b4 L-chains by Appella *et al.* (1971) and Fraser *et al.* (1972) are in agreement. Its partner (labelled 'V region' in Table 1) cannot be placed by any obvious homology, but, since this peptide is heterogeneous in both b4 and b5 rabbits, it is thought to come from the V region. Fraser *et al.* (1972) have evidence that this peptide lies near the middle of the V region, and Poulsen & Haber (1972) have shown that a disulphide bridge joins the V and C loops. Thus, from the above work plus the present study, it can be concluded that the extra disulphide bridge in rabbit κ -chains serves to join the V and C regions. It is noteworthy that, compared with the immunoglobulins of other animal species, there is an extra disulphide bridge in both the L-chain and the Fd portion of the H-chain (O'Donnell *et al.*, 1970), even though rabbits as a species evolved after the separation of the H-chain and L-chain genes.

In our previous work (Frangione & Lamm, 1970) on radioactively alkylated peptides we found only alanine at position 169 in b4 L-chains and only aspartic acid in the corresponding place in b5 L-chains. On this basis we proposed that an alanine/aspartic acid interchange is related to allotype. These findings have been confirmed in the present studies and also those of Fraser *et al.* (1972). However, Appella *et al.* (1971) did not find alanine at this position in a homogeneous b4 L-chain from an induced anti-*p*-azobenzoate antibody. Thus the exact relationship of residue position 169 to allotype is uncertain. Nevertheless normal κ -chains of the b4 and b5 allotypes can be differentiated by position 169 (see Fig. 1) as well as the C-terminal region (Appella *et al.*, 1969; Frangione, 1969).

In conclusion, we have investigated the pairing of the intrachain cysteine-containing peptides in rabbit κ -chains of two different allotypes and believe the disulphide bridges to be from the approximate positions 23–88, 134–194 and somewhere in the V region–171. This scheme agrees with that of Fraser *et al.* (1972), who studied b4 L-chains.

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