CORRELATION OF THE C-TERMINAL SEQUENCE OF RABBIT LIGHT CHAINS WITH ALLOTYPES

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Inherited variants of antigenic specificity (allotypes) have been found in all the immunoglobulins investigated. In rabbit IgG, correlation of amino acid content and peptide patterns with antigenic specificity has been reported for both heavy and light chain [1–5] and in the heavy chain correlation of specificity with amino acid sequence has been shown [6]. This supports the expected structural basis of the inherited variations, though a direct demonstration of antigenic specificity in the isolated variant peptides has not yet been observed.

We wish to report an extension of the sequence studies of the allotypic variants to the b locus in rabbit IgG - the locus controlling the structure of the kappa chain. The peptides containing easily reduced disulphide bonds - predominantly the interchain bonds have been examined.

Rabbit IgG (1% protein solution) was reduced in 0.5 M Tris-HCl pH 8.2 with 0.65 mM dithiothreitol for 60 min at 37° and alkylated by addition of iodo (1^{14C}) acetate (750,000 counts/min/μmole) to a concentration of 1.6 mM for 1 hr at room temperature. The protein was digested with pepsin at an enzyme-substrate ratio of 1:20 in 5% formic acid for 14 hr at 37°. Electrophoresis of the peptic digest on paper at pH 3.5 followed by autoradiography gave the pattern shown in fig. 1. A difference is shown which is unrelated to the allotype of the heavy chain but which shows some relation to the allotype of the light chain. Examination of digests of the separated chains showed that of the bands which varied 2, 4 and 5 were derived from the light chain.

Previous work [7] has shown that in human IgG the light-heavy interchain disulphide bond comes from

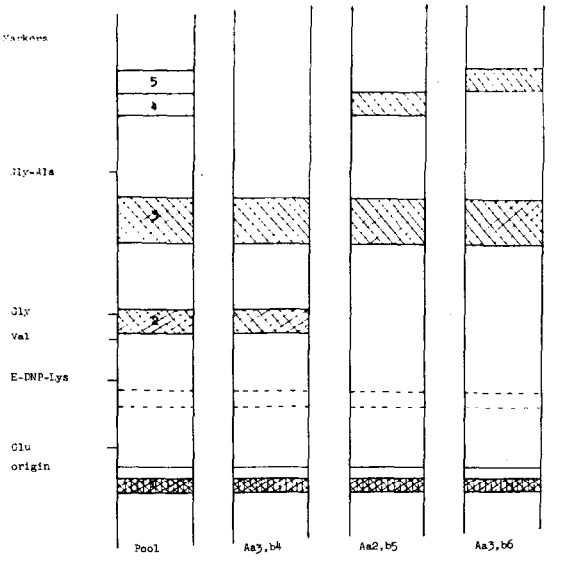

Fig. 1. Diagram showing radioactive bands after electrophoresis at pH 3.5 of a peptic digest of partially reduced and carboxymethylated rabbit γG of different allotypes. The intensity of the bands shown is as in the autoradiography.

the C terminal sequence of the light chain and the composition of peptide 2 (table 1) agrees with that of the C terminal peptide obtained from the light chain of IgG from pooled rabbit serum [8]. The N terminal amino acid and composition of peptide 3 agrees with the sequence containing labile cysteine residues near the centre of the heavy chain [9]. It appears that the light chains of allotype Ab5 and Ab6 contain different C terminal peptides to that of Ab4. When the sequences were determined (table 2) it became clear

Table 1
Carboxymethylated peptides isolated from a peptic digest of rabbit immunoglobulin G.

Peptide No.	Mob. at pH 6.5 relative + Asp (+1)	Composition	*N*-terminal
1	+0.68	Cys_2 Asp Thr_2 Ser_2 Pro_{2-3}	Pro
2(Aa3,b4)	+0.38	Cys Asp_2 Gly Arg	Asp
3	+0.10	Lys_3 Cys_2 Asp $Thr_{2.8}$ Ser_2 Glu Pro_5 Ala Val_2 $Met_{0.2}$ Leu	Lys
4(Aa2,b5)	−0.31	Cys Asp Ser Arg Lys	Ser
5(Aa3,b6)	−0.36	Cys Ser_2 Arg Lys	Ser

Table 2
Sequence of C-terminal peptides of rabbit light chain of different allotypes.

Peptide	Allotype	
2	Ab4	Asn-Arg-Gly-Asp-Cys
4	Ab5	Ser-Arg-Lys-Asn-Cys
5	Ab6	Ser-Arg-Lys-Ser-Cys

that each was distinct. A similar conclusion was reached in a recent publication [10], although the residue before the cysteine in b4 has been reported to be asparagine instead of aspartic acid. Current investigation of the peptides containing the more stable -S-S- bonds (presumably the intrachain disulphide bonds) again show differences between light chains of different allotype suggesting that allotype-related differences may be more extensive than those reported here in the C terminal pentapeptide, but the sequences concerned have not yet been established.

Either of the heavy chain peptides 1 and 3 may contain the cysteine residue linked to the light chain and direct evidence of involvement of peptide 3 has been obtained by the diagonal technique [11]. The peptide equivalent to peptide 1 could not be identified on the diagonal map, however, and it remains uncertain whether this peptide is involved in the heavy-light or heavy-heavy bond or is an exceptionally labile intrachain bond.

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