EXPRESSION OF ALLELIC AND NON-ALLELIC GENES IN IMMUNOGLOBULIN LIGHT CHAINS FROM HOMOZYGOUS RABBITS

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It was previously reported that there is a correlation between the *C*-terminal sequence of rabbit light (L) chains and allotypic specificity at the b locus [1, 2]. The question then arose whether L chain structural differences related to the b locus allotypes may exist in addition to those found at the *C*-terminal pentapeptide [2]. To answer this question selective peptides around half-cystine residues were isolated from rabbit L chains homozygous for the b4 and b5 alleles, and sequenced. The results indicate that in fact allotypic sequence differences are more extensive than those present at the *C*-terminus. It was also found that two amino acid sequences, which are not allotype-related, are present in each homozygous chain and that there is a highly variable section, probably around position 90.

Light chains were obtained after partial reduction and cold alkylation from the purified IgG of rabbits whose genotypes were a1, b4, or a1, b5 [3]. They were then completely reduced and carboxymethylated with iodo-^{14}C-acetate [4]. Peptic–tryptic peptides were purified by paper electrophoresis at different pH's and, when necessary, also by paper chromatography in BAWP [5, 6]. Six major radioactive carboxymethylated peptides were obtained from each L chain. The sequences of some of them (obtained by the dansyl-Edman techniques [5, 7]) are shown in table 1.

Comparison of the sequences of selected peptides from rabbit L chains homozygous for the b4 and b5 allotypes indicates:

1) There is a highly variable peptide present in both b4 and b5 L chains. (table 1: 1a1, 1a2, 1b1a, 1b1 and 1b1′). By homology with the sequences of L chains from other species the sequence Tyr–Tyr–Cys can be placed near positions 86–88 [8–10], a region which has been implicated in antibody specificity [11]. In Bence Jones proteins section 90–96 is one of the most variable regions [12, 13]. Studies of homogeneous rabbit antibodies, underway in a number of laboratories, can provide data on the relationship of this region to antibody specificity.

2) Two slightly different amino acid sequences, which appear not to be related to allotype, are present in each of two locations in each homozygous L chain (table 1, peptides 1e, 2b, 1a, 2b; 4c, 3b, 4b, 5b and 5a). This finding may result from the presence of V region subclasses analogous to those described in the L chains of other species [14–16] and suggests that in addition to the b locus, at least one other non-allelic gene is expressed in each homozygous L chain. We consider a contribution from b-negative L chains (i.e. λ chains, which are governed by the c locus [17, 18]) to be unlikely since b-negative chains form only about 15% of L chains in b4 and b5 rabbits [19] and our rabbits were not selected for homozygosity at the c locus. Hence λ peptides would not be expected to be detected by the techniques used. Consistent with this reasoning is our failure to detect the *C*-terminal peptide of the λ chain [2, 17].

3) The results in table 2 indicate that allotype related differences are more extensive than those previously reported at the *C*-terminal end. Both peptides 1b3a and 2a (table 2) were present in heterozygous rabbit L chains (b4/b5) while only one was present in homozygous animals. Several attempts to find both sequences in homozygous L chains have failed. Because of differences in mobility between peptides 1b3a and 2a (unpublished observations), diagonal electrophoresis at pH 3.5 makes the distinction between

Table 1
Sequences of carboxymethylated peptic - tryptic peptides of rabbit L chains of different allotype. Variant residues are in italics.

Peptide	Allotype	Sequence
1a1	b4	Tyr–Tyr–Cys–Gln–Gln–*Gly*–*Ser*–*Tyr*
1a2	b4	Tyr–Tyr–Cys–Gln–*Gly*–$\frac{Ser}{Ala}$
1b1a	b4	Tyr–Tyr–Cys–Gln–Gln–*Ser*–$\frac{Asn}{Gly}$
1b1	b5	Tyr–Tyr–(Cys, Gln, Gln, *Gly*) *Ser*–*Tyr*
1b1′	b5	Tyr–Tyr–(Cys, Gln, Gln, *Gly, Ser,* $Asp_{0.5}$, $Ala_{0.5}$)
1c	b4	Ile–*Val*–Cys
2b	b4	Ile–*Asn*–Cys
1a	b5	Ile–*Val*–Cys
2b	b5	Ile–*Asn*–Cys
4c	b4	Glu–Cys (*Ala,* Asp, Ala, Ala) Thr
3b	b4	Glu–Cys (*Asp,* Asp, Ala, Ala)
4b	b5	Glu–Cys–*Ala*–Asp–Ala (Ala, Thr)
5b, 5a	b5	Glu–Cys–*Asx*–Asx (Ala, Ala, Thr)
1b3a	b4	Thr–Pro–Glx–Asx–Ser–*Ala*–Asx (Cys, Thr)
2a	b5	Thr–Pro–Gln–Asn–Ser–*Asp*–Asp–Cys–Thr

Table 2
Correlation of amino acid sequence of rabbit L chains with allotype. Variant residues are in italics.

Peptide [2]	Allotype	Sequence	Peptide (see table 1)	Allotype	Sequence
2	b4	*Asn*–Arg–*Gly*–*Asp*–Cys	1b3a	b4	Thr–Pro–Glx–Asx–Ser–*Ala*–Asx(Cys, Thr)
4	b5	*Ser*–Arg–*Lys*–*Asn*–Cys	2a	b5	Thr–Pro–Gln–Asn–Ser–*Asp*–Asp–Cys–Thr

the b4 and b5 allotypes relatively easy.

Rabbit κ chains have certain structural characteristics which distinguish them from human and mouse κ chains. The major NH_2-terminal residue is Ala [20]; the majority contain 7 half-cystine residues instead of 5 [21]; and there is more than one amino acid substitution related to allotypic specificity in contrast to the Inv system of human κ chains [22]. It will be of interest to known whether any alloytype-related substitutions are in the V region.

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References

[1] E. Appella, J. Rejnek and R.A. Reisfeld, J. Mol. Biol. 41 (1969) 473.
[2] B. Frangione, FEBS Letters 3 (1969) 341.
[3] J.W. Prahl and R.R. Porter, Biochem. J. 107 (1968) 753.
[4] I.J. O'Donnell, B. Frangione and R.R. Porter, Biochem. J. 116 (1970) 261.
[5] B. Frangione and C. Milstein, J. Mol. Biol. 33 (1968) 893.
[6] C. Milstein, Biochem. J. 110 (1968) 652.
[7] W.R. Gray, Methods Enzymol. 11 (1967) 139, 469.
[8] N. Hilschmann and L.C. Craig, Proc. Natl. Acad. Sci. U.S. 53 (1965) 1403.
[9] K. Titani, E. Whitley, Jr. and F.W. Putnam, Science 152 (1966) 1513.
[10] W.R. Gray, W.J. Dreyer and L. Hood, Science 155 (1967) 465.
[11] S.J. Singer and N.O. Thorpe, Proc. Natl. Acad. Sci. U.S. 60 (1968) 1317.
[12] C. Milstein, FEBS Letters 2 (1969) 301.
[13] T.T. Wu and E.A. Kabat, J. Exptl. Med. 132 (1970) 211.
[14] L. Hood, W.R. Gray, B.G. Sanders and W.J. Dreyer, Cold Spring Harbor Symp. Quant. Biol. 32 (1967) 133.
[15] H.D. Niall and P. Edman, Nature 216 (1967) 262.
[16] C. Milstein, Nature 216 (1967) 330.
[17] E. Appella, R.G. Mage, S. Dubiski and R.A. Reisfeld, Proc. Natl. Acad. Sci. U.S. 60 (1968) 975.
[18] A. Gilman-Sachs, R.G. Mage, G.O. Young, C. Alexander and S. Dray, J. Immunol. 103 (1969) 1159.
[19] P. Bornstein and J. Oudin, J. Exptl. Med. 120 (1964) 655.
[20] R.F. Doolittle, Proc. Natl. Acad. Sci. U.S. 55 (1966) 1195.
[21] J. Rejnek, E. Appella, R.G. Mage and R.A. Reisfeld, Biochemistry 8 (1969) 2712.
[22] C. Baglioni, L.A. Zonta, D. Cioli and A. Carbonara, Science 152 (1966) 1517.