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Mechanism of Antibody Synthesis: Size Differences between Mouse Kappa Chains

Absrtact. *Structural analysis of immunoglobulin light chains has been carried out in an attempt to elucidate the genetic mechanisms involved in antibody synthesis. Analysis of two mouse kappa-chain proteins is almost complete. The differences are localized in one-half of the molecules, and do not reflect the operation of any one mutational mechanism. The peculiar character of the differences is discussed with reference to various theories of antibody formation. The finding that the two proteins differ in size is incompatible with certain proposed theories.*

The nature and extent of variation among immunoglobulin molecules has been studied as a means of testing various hypotheses concerning the mechanism of antibody formation. For this purpose, much attention has been focused on the proteins secreted by plasma-cell tumors, since these proteins can be readily isolated in a homogeneous form. Although these proteins are not functional antibodies made in response to a known antigenic stimulus, they appear to be truly representative of immunoglobulins in terms of their primary amino acid sequences. Most information has become available from structural studies of light-chain subunits of the K antigenic type, which have been obtained from tumors arising in mice and humans (*1–5*).

Such studies have shown that all proteins from within one species contain an extended region of structural identity (*6*, *7*) and that this region comprises approximately the carboxyl-terminal half of the molecule (*3*, this work). The variations are thus limited to the amino-terminal halves of the polypeptide chains.

Hitherto, the differences found in this region could be attributed to the random accumulation of point mutations, and they do not appear to have arisen by any particular genetic mechanism (*1*, *2*, *4*). The most extensive comparison yet made has been that between three human proteins (*2*, *3*), but the data were not sufficient to establish with certainty whether the proteins were of identical size—an important consideration for theories of antibody formation.

In this report we present a comparison between the amino acid sequences of two mouse K-chains [mouse Bence Jones proteins MBJ 41 and MBJ 70 produced by tumor lines MOPC 41 A and MOPC 70 E, respectively (*6*)] and the human K-chain Ag which has been investigated by Titani *et al.* (*2*). Probably MBJ 41 and the human proteins Ag and Cum each contain 214 amino acid residues (*2*, *3*). A slight doubt remains for MBJ 41 in that we have not yet been able to purify the large tryptic peptide containing residues 67 to 103, and hence we cannot assign a definite amino acid to position 87. The size of the human protein Roy (*3*) is not certain, but it probably contains 212 to 214 residues. Protein MBJ 70 has now been found to contain 218 residues, the four additional amino acids being inserted in the region of residues 27 and 28 (Fig. 1).

The evidence for this is quite unambiguous. The tryptic peptide defining residues 25 to 45 of MBJ 41 had the composition $Asp_{3.0}$, $Thr_{1.0}$, $Ser_{3.0}$, $Glu_{4.1}$, $Pro_{0.8}$, $Gly_{1.8}$, $Ala_{1.0}$, $Ilu_{1.8}$, $Leu_{2.1}$, $Lys_{1.1}$, Trp_1, a total of 21 (*8*). The corresponding peptide from MBJ 70 gave the analysis Asp_3, Met_1, $Ser_{3.5}$ $Glu_{4.0}$, $Pro_{3.0}$, $Gly_{2.0}$, $Ala_{1.1}$, $Val_{1.0}$, $Ilu_{1.0}$, $Phe_{1.9}$, $Lys_{2.0}$, Trp_1, a total of 25. The analyses were unchanged by further purification. Fragments were isolated after degradation of the peptides with chymotrypsin (MBJ 41) or subtilisin (MBJ 70) or cyanogen bromide (MBJ 70).

Amino acid compositions and sequences of these fragments were sufficient to establish the structures as shown in Fig. 1. The appropriate data for MBJ 70 are shown in Fig. 2. It should be noted that the -Lys-Pro bond is not susceptible to cleavage by trypsin, and hence the peptide from MBJ 70 contains two basic amino acids. The size difference between the two mouse proteins in this region is of special significance since it could not arise by a translational mechanism involving different readings of a single messenger RNA (*9*).

We have previously reported upon the sequences of the first six residues of a number of mouse and human K-chains (*1*) and established that the same

Table 1. Comparison of related pairs of proteins and genes. The comparison is made by (i) amino acid (AA) substitutions; (ii) the minimum number of nucleotide base changes required to account for these substitutions in terms of point mutations, with the use of the coding assignments of Brimacombe *et al.* (*11*); (iii) classification of base changes as transversions or transitions.

Chains		AA Residues	Changes between related genes: Differences No.	Differences %	Minimum base changes 1	2	3	Transitions	Transversions
				K light chains					
Variable 1–107	Mouse 41	107	42	40	27	12	1	32	17
	Mouse 70	111							
Common 108–214	Mouse	107	45	42	32	13	0	20	28
	Human	107							
				Hemoglobin					
	Human β	146	37	26	28	9	0	28	16
	Human γ	146							

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MOUSE 41   Asp.Ilu.Gln.Met.Thr.Gln.Ser.Pro.Ser.Ser.Leu.Ser.Ala.Ser.Leu.Gly.Glu.Arg.Val.Ser.Leu.Thr.Cys.Arg.Ala.
MOUSE 70   Asp.Ilu.Val.Leu.Thr.Gln.Ser.Pro.Ala.Ser.Leu.Ala.Val.Ser.Leu.Gly.Gln.Arg/Ala.Thr.Ilu.Ser.Cys.Arg/Ala.
HUMAN Ag   Asp.Ilu.Gln.Met.Thr.Gln.Pro.Ser.Ser.Ser.Leu.Ser.Ala.Ser.Val.Gly.Asp.Arg.Val.Thr.Ilu.Thr.Cys.Gln.Ala.

41  Ser.Gln.— — — — Asx.Ilu.Gly.Ser.Leu.Ser.Asx.Trp.Leu.Glx.Glx(Gly,Pro,Asx,Thr,Glx)Ilu.Lys.Arg.Leu.Ilu.Tyr.Ala.
70  Ser.Glu.Ser.Val.Asx.Asx.Ser.Gly.Ilu.Ser.Phe.Met.Asn(Trp,Phe,Glx)Glx.Lys.Pro.Gly.Glx.Pro.Pro.Lys/Leu.Leu.Ilu.Tyr.Ala.
Ag  Ser.Gln.— — — —(Asx,Ilu,Asx,Ser,Phe)Leu.Asn.Trp.Tyr.Gln.Gln.Gly.Pro.Lys.Lys.Ala.Pro.Lys.Ilu.Leu.Ilu.Tyr.Asp.

41  Thr.Ser.Ser.Leu.Asx.Ser.Gly.Val.Pro.Lys.Arg.Phe.Ser.Gly.Ser.Arg.Ser.Gly.Ser.Asp/Tyr.Ser.Leu.Thr/Ilu.Ser.Ser.Leu/Glu.
70  Ala.Ser.Asn.Gln.Gly.Ser.Gly.Val.Pro.Ala.Arg/Phe.Ser.Gly.Ser.Gly.Ser.Gly.Thr.Asp.Phe.Ser.Leu.Asn.Ilu.His.Pro.Met.Glx.
Ag  Ala.Ser.Asn.Leu.Glu.Thr.Gly.Val.Pro.Ser.Arg.Phe.Ser.Gly.Ser.Gly.Phe.Gly.Thr.Asp.Phe.Thr.Phe.Thr.Ilu.Ser.Gly.Leu.Gln.

41  Ser.Glu.Asp.Phe.Val.Asp.Tyr/ ? /Cys.Leu.Gln.Tyr.Ala.Ser.Ser.Pro.Trp.Thr.Phe.Gly.Gly.Gly.Thr.Lys.Leu.Glu.Ilu.Lys.Arg.
70  Glx.Asx.Asx.Thr.Ala.Met.Tyr.Phe.Cys.Glx.Glx.Ser.Lys/Glu.Val.Pro.Trp.Thr.Phe.Gly.Gly.Gly.Thr.Lys.Leu.Glu.Ilu.Lys/Arg
Ag  Pro.Glu.Asp.Ilu.Ala.Thr.Tyr.Tyr.Cys.Gln.Gln.Tyr.Asp.Thr.Leu.Pro.Arg.Thr.Phe.Gly.Gln.Gly.Thr.Lys.Leu.Glu.Ilu.Lys.Arg.

41  Ala.Asx.Ala.Ala.Pro.Thr.Val.Ser(Ilu,Phe,Pro,Pro,Ser,Ser)Glu.Gln.Leu.Thr.Gly(Gly,Ser,Ala,Ser)Val.Val.Cys.Phe.Leu.Asn.
70  (Ala,Asx,Ala,Ala,Pro,Thr,Val,Ser,Ilu,Phe,Pro,Pro,Ser,Ser,Glx,Glx,Leu,Thr,Gly,Gly,Ser,Ala,Ser,Val,Val)Cys.Phe(Leu,Asx,
Ag  Thr.Val.Ala.Ala.Pro.Ser.Val.Phe.Ilu.Phe.Pro.Pro.Ser.Asn.Glu.Gln.Leu.Lys.Ser.Gly.Thr.Ala.Ser.Val.Val.Cys.Leu.Leu.Asn.

41  Asn.Phe.Tyr.Pro.Lys.Asp.Ilu.Asn.Val.Lys.Trp.Lys.Ilu.Asp.Gly.Ser.Glu.Arg.Gln.Asx.Gly.Val.Leu(Glx,Ser,Asx,Thr,Asx,Trp)
70  Asx,Phe,Tyr,Pro)Lys/Asp.Ilu.Asn.Val.Lys/Trp.Lys.Ilu(Asp,Gly,Ser,Glu)Arg/(Glx,Asx,Gly,Val,Leu,Glx,Ser,Asx,Thr,Asx,Trp,
Ag  Asn.Phe.Pro.Tyr.Arg.Glu.Ala.Lys.Val.Gln.Trp.Lys.Val.Asp.Asn.Ala.Leu.Gln.Ser.Gly.Asn.Ser.Gln.Glu.Ser.Val.Thr.Glu.Gln.

41  Asp.Ser.Lys.Asp.Ser.Thr.Tyr.Ser.Met.Ser.Ser.Thr.Leu.Thr.Leu.Thr.Lys.Asx.Glx.Tyr.Glx.Arg.His.Asx.Ser.Tyr.Thr.Cys.Glx.
70  Asp,Ser)Lys/Asp(Ser,Thr,Tyr,Ser,Met,Ser,Ser,Thr,Leu,Thr,Leu,Thr)Lys/Asx(Glx,Tyr,Glx)Arg/His(Asx,Ser)Tyr.Thr.Cys.Glx
Ag  Asp.Ser.Lys.Asp.Ser.Thr.Tyr.Ser.Leu.Ser.Ser.Thr.Leu.Thr.Leu.Ser.Lys.Ala.Asp.Tyr.Glu.Lys.His.Lys.Val.Tyr.Ala.Cys.Glu.

41  Ala.Thr.His.Lys.Thr.Ser.Thr.Ser.Pro.Ilu.Val.Lys.Ser.Phe.Asn.Arg.Asn.Glu.Cys
70  (Ala,Thr,His)Lys/Thr(Ser Thr,Ser,Pro,Ilu,Val)Lys/Ser.Phe.Asn.Arg.Asn.Glu.Cys
Ag  Val.Thr.His.Gln.Gly.Leu.Ser.Ser.Pro.Val.Thr.Lys.Ser.Phe.Asn.Arg.Gly.Glu.Cys
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Fig. 1. Amino acid sequences of mouse and human K-chains. Residues are numbered on the basis of a polypeptide chain length of 214 amino acid residues rather than of 212 as was used earlier (2). The sequence of the human protein Ag is taken from Putnam *et al.* (2). Diagonal bars after certain residues in the mouse proteins indicate that the alignment of peptides is derived from analogy with the other mouse protein.

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T–1  Ala(Ser,Glu,Ser,Val,Asx,Asx,Ser,Gly,Ilu,Ser,Phe,Met,Asn,Trp, Phe,Glx,Glx,Lys,Pro,Gly,Glx,Pro,Pro)Lys
S–1  Ala. Ser.Glu.Ser
S–2                Val.Asx.Asx.Ser
S–3                          Gly.Ilu.Ser.Phe
S–4                                    Met.Asn
S–5                                         (Trp,Phe,Glx)
S–6                                                    Glx.Lys.Pro.Gly.Glx.Pro.Pro.Lys
S–7  (Ala,Ser,Glu,Ser,Val,Asx,Asx,Ser)
S–8                                                    Lys.Pro.Gly.Glx.Pro.Pro.Lys
C–1  (Ala,Ser,Glu,Ser,Val,Asx,Asx,Ser,Gly,Ilu,Ser,Phe)Hsr

Sequence
Ala.Ser.Glu.Ser.Val.Asx.Asx.Ser.Gly.Ilu.Ser.Phe.Met.Asn(Trp,Phe,Glx)Glx.Lys.Pro.Gly.Glx.Pro.Pro.Lys
25  27  27a 27b 27c 27d 28  30  35  40  45
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Fig. 2. Structure of tryptic peptide from MBJ 70. T–1, original tryptic peptide. S–1 to S–6, major components produced by digestion of T–1 with subtilisin; S–7 and S–8 were minor components present in the same digest. C–1, peptide produced by cleavage of T–1 with cyanogen bromide, which converts Met to homoserine (Hsr).

variations occur in both species. From a comparison of the amino-terminal halves of the proteins shown in Fig. 1, it will be seen that there are more differences between the two mouse proteins than between MBJ 41 and Ag.

Table 1 contains a summary of the changes observed between the amino-terminal halves of MBJ 41 and MBJ 70, between the carboxyl-terminal halves of mouse and human K-chains, and also between the β- and γ-chains of human hemoglobin. All three cases show a similar pattern of changes. In view of this similarity, the size difference between MBJ 41 and MBJ 70, and the failure to find a single genetic mechanism to account for all of the observed changes, we feel that the evidence points overwhelmingly to the conclusion that the changes have arisen by the accumulation of random mutations of the various types which normally account for the evolution of protein molecules. There is much less variation expressed at individual positions (*1*) than would be expected to arise by any single mutation mechanism or by a random process, and it is clear that strong selective forces must operate to produce this restriction.

It is not yet possible to say whether the mutation and selection process take place over the normal evolutionary time scale, or during the somatic differentiation of the immune system in each individual. In either case, the available structural information places some remarkable constraints upon the genetic mechanisms involved in the production of light-chain molecules (*10*).

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8. Abbreviations used are Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Ilu, isoleucine; Leu, leucine; Lys, lysine; Trp, tryptophan; Met, methionine; Val, valine; Phe, phenylalanine; Tyr, tyrosine; Cys, cystine; Arg, arginine; Asn, asparagine; Asx, aspartic acid or asparagine; Hsr, homoserine; Glx, glutamic acid or glutamine; and Gln, glutamine.
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Protein Uptake in Multivesicular Bodies in the Molt-Intermolt Cycle of an Insect

Abstract. *Plant peroxidase injected into the hemocoel is taken up in granules by almost all tissues. These granules may become multivesicular bodies or isolation bodies which later breakdown. There is most uptake and breakdown at times in the molt-intermolt cycle when cells are engaged in active syntheses.*

In the course of ultrastructural studies on changes during the molt-intermolt cycle it was found that almost all tissues contained dense, membrane-bound granules, varying in size from 0.1 to 10μ in diameter. Intensive work failed to show any gross connection with Golgi complexes. The granules did not contain acid phosphatase and could be distinguished from autophagic vacuoles and residual bodies (*1*) resulting from autophagy. In addition to the dense granules, the tissues also contained structures similar to multivesicular bodies (*2*) and to what are now recognized as a variety of intermediates. The origin of these granules and of the multivesicular bodies has been determined in studies on the fate of plant peroxidase injected into the hemocoel. The granules and multivesicular bodies contain protein taken up from the blood.

There is evidence for fairly specific uptake of blood proteins by developing oocytes to form yolk (*3*) and by salivary glands for the secretion that they extrude (*4*). The protein granules of the fat body which appear at metamorphosis are also sequestered blood protein (*5*). The visualization of plant peroxidase taken up by almost all tissues demonstrates the ubiquity of protein utilization in a most dramatic way.

Events in the 8 days (192 hours, S.D. 13) of the last (fifth) larval stadium of *Calpodes ethlius* have been precisely timed and can be predicted to within a few hours. During the fifth stadium, larvae gain in weight from about 0.5 to 1.5 g. Batches of larvae were injected at various stages with peroxidase (0.5 mg/g, Worthington) freshly prepared in 0.1 ml insect Ringer, for incorporation periods of 2 to 24 hours. The technique is similar to that extensively developed for vertebrate studies by Straus (*6*). The larvae were fixed, by standard procedures, in glutaraldehyde as if for electron microscopy (*7*). Tissues were incubated at room temperature for 10 minutes in a mixture of diaminobenzidine (0.03 percent) and hydrogen peroxide (0.01 percent) in tris(hydroxymethyl)aminomethane buffer (*p*H 7.6) and 10 percent sucrose (*8*). They were then either mounted for light microscopy or treated with osmium tetroxide for electron microscopy.

After the benzidine reaction the granules of sequestered protein appear dark brown in light microscopy (Figs. 1 and 2). Unstained sections viewed with the electron microscope show the peroxidase with extra density from the deposition of osmium. The enzyme is localized in granules and multivesicular bodies. The peroxidase could itself stimulate the tissues to sequester it as part of a "cleaning up" process. However, stained sections of larvae without peroxidase show a complete sequence from newly formed protein granules to multivesicular bodies with dense contents (Fig. 3). The similarity of the ultrastructure in tissues exposed and not exposed to peroxidase suggests that the uptake of foreign protein is qualitatively similar to the natural event.

Using this technique, we have studied protein sequestration in a number of tissues during the molt-intermolt cycle (Fig. 4). In most of the epidermis, protein granules are minute or absent until 60 to 70 hours after ecdysis, when deposition of intermolt endocuticle is greatly accelerated. This corresponds to the time when the prothoracic gland has been activated by the brain for