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THE AMINO-TERMINAL AMINO ACID SEQUENCES OF RABBIT IMMUNOGLOBULIN LIGHT CHAINS*

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In their study of two homogeneous but nonidentical human Bence-Jones proteins, Hilschmann and Craig¹ established that the carboxy-terminal halves of these molecules were essentially identical, whereas the amino-terminal halves had a substantial number of amino acid differences. The data of Titani *et al.*² on a third Bence-Jones protein substantiated these findings, and the elegant disulfide mapping studies of Milstein³ suggested that the concept of a "variable amino-terminal region" and a "constant carboxy-terminal area" also applied to normal human γ -globulin. On the other hand, detailed inspection of the available sequence data^{1, 2} for the amino-terminal halves of the Bence-Jones proteins suggested that the amino acid sequences were not at all random and hinted that the possible amino acid variations at any particular position in the amino-terminal region might be very limited.⁴ Indeed, rabbit light chains were thought to end predominantly in a single amino acid,⁵ and early structural studies⁶ on normal rabbit γ -globulin and a wide variety of purified rabbit antibodies indicated a unique sequence at the amino-terminal end: alanyl-leucyl-valyl-aspartyl-glutamine.

It has been implicit in the structural studies on Bence-Jones proteins that the light chains of immunoglobulins, and in particular the variable portions of them, play a role in determining antibody specificity. Light chains, as well as heavy chains, have indeed been implicated in the binding specificity of antibodies,⁹ and the recent suggestion¹⁰ that light and heavy chains have evolved from a common ancestral molecule by a series of gene duplications has further convinced us that the participation of both chains is fundamental to immunospecificity. With these considerations in mind, we decided to investigate the amino-terminal sequences of light chains from rabbit anti-dinitrophenyl (anti-DNP) antibodies by the Edman¹¹

direct degradation scheme. The array of amino acids which we found differs substantially from the unique sequence previously reported.⁶

Subsequently, Hood *et al.*¹² have applied the same Edman method to a variety of mouse and human myeloma light chains (Bence-Jones proteins) with considerable success. They have found that the variability at each of the first six positions of these light chains is extremely restricted, and that the mouse and human patterns were virtually indistinguishable in this region. These studies prompted us to re-examine our unpublished data on the rabbit anti-DNP light chains and to extend our studies to normal rabbit γ -globulin light chains as well. Our results confirm the findings of Hood *et al.*¹² with regard to the limited variability in the amino-terminal region of light chains, but at the same time demonstrate that the rabbit chain sequences are readily distinguishable from their mouse and human counterparts.

Materials and Methods.—Pooled rabbit anti-DNP antibody was prepared as described previously;¹³ rabbit γ -globulin was obtained from Pentex. The immunoglobulin preparations were subjected to mild reduction and the light chains separated from the heavy chains according to the methods of Fleishman *et al.*⁵ The Edman degradations were carried out using the three-cycle modification.¹⁴ About 10 mg of light chain were coupled with phenyl isothiocyanate at pH 9.0–9.2 for 60 min at 40°. The preparations were washed thoroughly with benzene or butyl acetate, suspended in water and freeze-dried overnight. After further washing with ethyl acetate, the phenyl thiocarbamyl-proteins were cleaved with trifluoroacetic acid. Thiazolidones were extracted with ethylene chloride and converted to their corresponding phenyl thiohydantoin (PTH-) according to the procedure of Ilse and Edman.¹⁵ The aqueous phases containing water-soluble PTH-amino acids (arginine and histidine) were examined only in the first two cycles of any degradation. Quantitative studies on the PTH-amino acids eluted from paper chromatograms were performed by the Sjöquist procedure.¹⁶ Qualitative identification of PTH-amino acids was made on Eastman silica gel thin-layer chromatograms. The classical “D, E, F” paper chromatographic systems of Edman and Sjöquist¹⁷ were supplemented with a chloroform/formic acid (100/5) system.

Results.—The PTH-amino acids identified in the course of six Edman cycles on normal rabbit γ -globulin and anti-DNP light chains are summarized in Table 1. The results from the two types of preparation were qualitatively similar throughout. Quantitatively, however, the terminal amino acid compositions of light chains from

TABLE 1
PTH-AMINO ACIDS IDENTIFIED AT AMINO-TERMINAL END OF IMMUNOGLOBULIN LIGHT CHAINS

Light chain source	Position in Peptide Chain*					
	1	2	3	4	5	6
Rabbit γ -globulin	ALA (Ilu, Asp, Glu)	VAL (Leu)	VAL LEU (Gln/Glu)	VAL (Gln/Glu)	GLN	GLN (Thr, Ala)
Rabbit anti-DNP	ALA ILU (Asp, Glu)	VAL	VAL LEU (Gln/Glu)	VAL (Gln/Glu)	GLN ALA	GLN ALA (Thr)
Mouse and human†	ASP GLU	ILU	VAL GLN	VAL LEU MET	THR	GLN

* Positions numbered from amino-terminal end. PTH-amino acids shown in parentheses occurred in small amounts.

† Summarized from Hood *et al.*¹²

three different anti-DNP pools were significantly and reproducibly different from the normal γ -globulin preparation (Table 2). In addition, quite obvious differences in the relative intensities of several spots were detected at positions 5 and 6 between the anti-DNP and normal γ -globulin chains.

Position 1: In all cases, alanine was the major amino-terminal residue (Fig. 1a). In addition, however, a significant amount of isoleucine was always found. When anti-DNP light chains were compared with γ -globulin light chains, the amount of alanine decreased significantly and the amount of isoleucine increased threefold

FIG. 1.—Ultraviolet photographs of thin-layer chromatograms, solvent "D" of Edman and Sjöquist.¹⁷ All descriptions listed left to right: (a) standard PTH-mixture I, PTH-Leu, PTH-Ilu, γ '-step 1, DNP-step 1, DNP'-step 1, PTH-Ala, standard PTH-mixture II; (b) standard PTH-mixture I, γ -step 5, PTH-val, diphenylthiourea, γ '-step 2, DNP-step 2, DNP'-step 2, standard PTH-mixture II; (c) standard PTH-mixture I, γ -step 6, PTH-Val, γ '-step 3, DNP-step 3, DNP'-step 3, PTH-Leu, standard PTH-mixture II; (d) standard PTH-mixture I, PTH-Pro, γ -step 8, PTH-Val, γ '-step 5, PTH-Met, DNP-step 5, standard PTH-mixture II. PTH-glutamine and other less hydrophobic PTH-amino acids remain at the origin in this solvent. γ - and γ '-represent two experiments on the same original batch of normal rabbit γ -globulin light chains. DNP and DNP' represent light chains from two different batches of rabbit anti-DNP antibody.

TABLE 2
QUANTITATIVE STUDIES ON AMINO-TERMINAL AMINO ACIDS OF RABBIT γ -GLOBULIN
AND ANTI-DNP LIGHT CHAINS

Light chain source		Moles/25,000 gm*			Step 2 PTH-Val
		PTH-Ala	PTH-Ilu	(Ala/Ilu)	
γ -Globulin	Expt. 1	0.38	0.06	(6.3)	0.24
	Expt. 2	0.40	0.07	(5.7)	0.21
Anti-DNP	Batch 1	0.23	0.15	(1.5)	0.20
	Batch 2	0.30	0.18	(1.7)	0.24
	Batch 3	0.21	0.14	(1.5)	0.20

* Air-dry weights. Values uncorrected for procedural losses. Calculations based on $E_{\text{Ala}} = 16,000$, $E_{\text{Ilu}} = 17,000$, and $E_{\text{Val}} = 16,500$. Elutions after paper chromatography on Sjöquist solvent I (ref. 16). Small amounts of PTH-aspartic acid and glutamic acid appeared in step 1 in all cases also. The anti-DNP batches were prepared from different pools of rabbit sera.

(Table 2). The PTH-isoleucine was distinguished from PTH-leucine by repeated runs on solvent D on both paper and thin-layer chromatography. Trace amounts of PTH-aspartic acid and PTH-glutamic acid were generally detected also. The over-all recovery of PTH-amino acids was about 50 per cent (uncorrected for procedural losses) and does not rule out the possibility that some rabbit light chains have no terminal amino group.

Position 2: The only amino acid found at position 2 in the anti-DNP light chains was valine. The γ -globulin light chains also contained mainly valine, but a small amount of leucine was also detected (Fig. 1b). The quantitative values of the PTH-valine recovered at position 2 were essentially the same in the two different types of preparation (Table 2).

Positions 3 and 4: Both preparations of light chain gave the same results at these positions. In all cases, valine was the major amino acid found. An appreciable amount of PTH-leucine or isoleucine was detected at position 3 (Fig. 1c), but none at position 4. In addition, a substantial amount of a derivative which gave the appearance of PTH-glutamine on solvent E was found in both positions. When this material was rechromatographed in the chloroform/formic system, however, it corresponded to PTH-glutamic acid. This behavior was in contrast to the PTH-glutamine identified at positions 5 and 6.

Positions 5 and 6: The predominant amino acid at positions 5 and 6 in rabbit light chains is glutamine. The PTH-glutamine gave a striking yellow color on solvent E and was verified in the chloroform/formic acid system. In both solvents, it chromatographed perfectly with authentic PTH-glutamine. The anti-DNP light chains were different from the γ -globulin light chains in that they also contained a significant amount of alanine at these positions. The γ -globulin light chains gave no evidence of alanine at position 5 and only a small amount at position 6. The intensity of PTH-glutamine spots was greater at these positions in the case of the γ -globulin preparations than it was for the anti-DNP chains. In addition, a small amount of PTH-threonine was found in all cases at position 6.

One of the normal γ -globulin preparations was carried through a total of nine Edman cycles. The picture at position 7 was confusing, although the multiple spot pattern obtained is often characteristic of PTH-serine. At position 8, PTH-proline was detected for the first time. It was accompanied by substantial amounts of PTH-alanine and PTH-valine. At the ninth position, PTH-glycine appeared and was

confirmed by its characteristic pink spot on solvent D. PTH-alanine, PTH-proline, and PTH-valine were present also.

Discussion.—The pattern of amino acids obtained at the amino-terminal end of pooled rabbit light chains¹⁸ is at once similar to and different from that obtained by Hood *et al.*¹² on mouse and human systems. For example, two major terminal residues have been identified in the rabbit system (alanine and isoleucine), but they are different from the two predominant terminal residues found in human γ -globulin light chains (aspartic acid and glutamic acid). Only one major amino acid is found at position 2 in both systems, but in the case of the rabbit it is valine, whereas in the mouse and human systems it appears to be isoleucine. Positions 3–6 appear to be somewhat variable in the rabbit light chains, but the amino acids detected are often the same ones which occur in the mouse and human light chains in that region (especially valine, leucine, and glutamine) (Table 1).

Dreyer and Bennett¹⁹ have proposed that multiple germ line genes are responsible for the specificity regions of immunoglobulins. The data of Hood *et al.*¹² on the amino-terminal sequences of mouse and human light chains are regarded as being compatible with such a scheme.¹² It seems clear that the data presented here for rabbit light chains are inconsistent with such an hypothesis. For example, the isoleucine at position 2 in mouse and human light chains is thought to be “invariable.” If this isoleucine corresponds to the similarly “invariable” valine at position 2 in the rabbit light chains, then one must presume that the amino acid replacement has been effected by a single base substitution in the genetic material. If “multiple germ line genes” exist, then we must submit that the same base substitution has occurred in *all* the “multiple specificity genes” somewhere on the evolutionary tree between rabbits on the one hand, and mice and humans on the other. It would seem very unlikely that the same amino acid replacement would have occurred in each of the proposed multiple genes. Even if the amino-terminal region of the rabbit light chains is not directly comparable to the amino-terminal region of the mouse and human light chains, as might happen if the rabbit light chains are not the same length as mouse and human light chains, then once again one must presume that the difference is the result of a deletion or insertion somewhere on the evolutionary tree between rabbits and the other two mammals. It would be even more unlikely that the same deletion and/or insertion would have occurred in all of a large number of “specificity genes.”

It seems significant that the purified antibody preparation yielded light chains which were no less variable in their amino-terminal sequences than the normal rabbit γ -globulin. Electrophoretic evidence²⁰ for a degree of light chain selection as a function of antibody specificity has been presented for guinea pig systems. On the other hand, the anti-DNP light chains used in the present study have previously been shown to be electrophoretically heterogeneous and the heterogeneity implicated with the binding region.²¹ Now we have direct evidence for amino acid variability at the amino-terminal ends of light chains from a specific rabbit antibody.

With regard to the large quantitative differences in the amounts of alanine and isoleucine at the amino-terminals of rabbit normal γ -globulin and anti-DNP light chains, it would be premature to conclude that there is a connection here with some selective mechanism associated with immunospecificity. A definitive study would have to compare preimmune γ -globulin with antibody from the same individuals.²²

The fact remains, however, that the two different preparations of rabbit immunoglobulin light chains (i.e., Pentex normal γ -globulin on the one hand, and three pools of anti-DNP antibody on the other) do differ significantly with regard to the amounts of certain amino acids found at definite positions in the amino-terminal region.

Summary.—Amino acids occupying the first six positions of rabbit immunoglobulin light chains have been identified. The amino acid sequence is not as unique as had previously been supposed. Comparison with data reported for mouse and human immunoglobulin light chains substantiates the notion of a limited variability at each position near the amino-terminal end, but casts considerable doubt on a recently proposed theory of immunogenesis. Rabbit anti-DNP light chains were qualitatively similar to normal rabbit γ -globulin light chains in the amino-terminal region, but quantitative differences were readily apparent.

Note added in proof: It should be emphasized that the only light chains susceptible to Edman degradation in these experiments are those with free α -amino groups. Hood (personal communication) has now found that human λ -type chains generally have blocked amino-terminals, probably of the pyrrolidone carboxylic acid type. It is the class of human light chains designated κ which invariably has aspartic acid or glutamic acid at the amino-terminal.

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A MOLECULAR MODEL FOR GENE REPRESSION

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In their original formulation of the regulator gene hypothesis, Jacob and Monod suggested that the repressor might be a polynucleotide possessing a specific base sequence which was complementary to an operator site.¹ However, more recent studies have conclusively shown that the repressor is a protein, composed of subunits, possessing allosteric properties.²⁻⁵ A peculiar property of the repressor is its instability under conditions of growth. This has prompted the suggestion that the active repressor could be a protein-messenger RNA complex, the messenger fraction accounting for its growth lability.⁴

The purpose of this communication is to suggest a plausible molecular model for gene repression, based on the fundamental hypothesis that the repressor is a *ribonucleoprotein* capable of interacting with an operator site in the deep groove of the DNA helix, using an alternate base-pairing configuration. Evidence for the existence of this alternate pairing configuration has been provided by studies on certain polynucleotide complexes and crystallographic studies of purine-pyrimidine crystalline complexes.

The Model.—The model, which is represented by the diagrams in Figures 1 and 2, involves the following features.

(1) Stretches of purine sequences in the double helical DNA structure can accommodate short pyrimidine-containing RNA oligonucleotides in the deep groove of the helix. This is accomplished by means of an alternate base-pairing configuration, one involving hydrogen bonding with the imidazole nitrogen, N-7, of adenine and guanine. *The Watson-Crick pairing rules are maintained* (adenine = uracil, guanine = cytosine), *but the geometry of the interaction is different* (Fig. 1).

(2) The regulator gene and the operator gene contain segments of DNA which have similar (or perhaps identical) stretches of *purine-containing sequences*.

(3) The product of the regulator gene (or genes) is a ribonucleoprotein which is composed of (a) at least part of the *messenger RNA transcribed from the regulator gene DNA*, (b) the protein coded by this gene (or genes). The messenger RNA portion must contain a stretch of pyrimidines which is complementary to a stretch of purines on the operator site.