THE *N*-TERMINUS OF LIGHT CHAINS FROM RABBIT ARSONIC ANTIBODY*

K.J.FRASER** and P.EDMAN
St. Vincent's School of Medical Research, Melbourne, Vic. 3065, Australia

Received 19 January 1970

1. Introduction

There have been few studies of the amino acid sequence of light chains isolated from specific antibody molecules. In contrast to light chains from myeloma proteins, which have a unique amino acid sequence [1–3], light chains from specific antibodies to dinitrophenol groups have been reported to be heterogeneous in amino acid sequence [4, 5]. More recently some degree of amino acid sequence restriction has been demonstrated in the first three *N*-terminal positions of light chains obtained from IgG rabbit antibodies to the group specific carbohydrates of groups A and C streptococci [6].

We report here a study of the *N*-terminal amino acid sequence of light chains isolated from rabbit antibodies to the hapten *p*-azobenzenearsonate. The application of the sequenator procedure [7] has facilitated the determination of amino acid residues at the first ten *N*-terminal sequence positions. Comparison with results obtained from pooled normal light chains revealed markedly less heterogeneity in the antibody light chain preparation. The results also suggest a further reductions of sequence heterogeneity if one assumes the presence of two major classes of light chain which differ, near the terminus, by the presence or absence of an *N*-terminal alanine residue.

* Antibody to the hapten *p*-azobenzenearsonate will be referred to in this paper as arsonic antibody.

** Present address: Department of Medicine, University of Melbourne., St. Vincent's Hospital, Melbourne, Vic. 3065, Australia.

2. Materials and methods

New Zealand white rabbits (Tillside Rabbit Stud, Yanderra, N.S.W.) were used. All rabbits used were homozygous at the b locus and of b4 allotypic specificity.

The preparation of rabbit antibody to *p*-azobenzenearsonate was obtained according to established procedures [8].

Non-immune IgG was obtained from pooled rabbit sera using ammonium sulphate precipitation and subsequent chromatography on a DEAE cellulose column equilibrated with 0.0175 M phosphate buffer pH 6.3 [9].

The reduced and alkylated light chains [10] were separated from the heavy chains by chromatography on G-100 Sephadex at 4°C using 1 M propionic acid as the eluant.

The amino acid content of the sequence positions at the *N*-terminus of the light chains was determined by the phenylisothiocyanate technique, either in the sequenator [7] or the manual version [11].

3. Results

The results are presented in table 1. There is clearly much less heterogeneity in the sequence positions at the *N*-terminus of light chains from the arsonic antibody as compared to the pooled light chain preparation. From positions 1 to 10 the results became less clear, although at positions 11–15 two major components were still present, but minor amounts of other amino acids were also found. Comparison with the results from the pooled preparation showed in most cases that the amino acids

Table 1
Amino acids in the first *N*-terminal positions of rabbit light chains.[1]

	1									10
Arsonic antibody:	Ala	Asp	Ile	Val	Met	Thr	Gln	Thr	Pro	Ala
	Asp	Val	Val	Met	Thr	Gln	Thr	Pro	Ala	Ser
		Glu					*Pro*			
Normal pool:	Ala	Val	Ile	Val	Val	Thr	Gly			
	Asp	Tyr	Val	Thr	Gln	Gln	Val			
		Gln		*Met*	*Thr*	*Val*	*Gln*			
				Ser	*Ser*	*Ser*	*Thr*			
							Pro			
							Thr			

[1] Minor components in italics.

found in the antibody preparation are present in the pooled preparation at the corresponding position. The only exceptions are the presence of aspartic acid at position 2 and methionine at position 5 of the antibody preparation. Quite clearly, however, the converse situation does not hold.

4. Discussion

The presence of obvious quantitative differences in the amounts of amino acids found at corresponding positions of the arsonic antibody preparation and the pooled preparation immediately suggests selection of a subpopulation of light chains by the *p*-azobenzenearsonate antigen. The finding of aspartic acid at position 2 and methionine at position 5 indicates that some of the light chains from the specific arsonic antibody are either not represented in the normal pool preparation or that these amino acids are present in the pooled preparation in amounts too small for the PTH derivatives to be identified using thin layer chromatography. Whatever the role of the light chain in the mechanism of antibody specificity it is apparent that different antigens do induce the synthesis of light chains differing in *N*-terminal amino acid content when compared with the normal pool. This may represent the selection of a particular subclass of light chain necessary for the formation of the antibody combining site [6].

The overlapping pattern of amino acid residues in the arsonic antibody preparation (table 1) suggests the presence of polypeptide chains of differing lengths; this phenomenon has previously been reported in human fibrinopeptides [11], human placental lactogen [12] and ox growth hormone [13]. Such situations could result from the action of serum endopeptidases [14] or from genetic deletions. An attempt was made to establish such a phase shift in the arsonic antibody preparation by cleavage of the methionine containing bonds. However the degree of cleavage was slight in accord with earlier observations on the cyanogen bromide cleavage of -Met-Thr- bonds [15, 16].

Further studies of *N*-terminal amino acid content of specific antibody light chains are indicated in order ro determine the relationship of amino acid changes to the antigenic determinant and also the presence or absence of chain variations in this region.

Acknowledgements

We wish to thank Dr. R.A.Reisfeld, National Institutes of Health, Bethesda, Maryland, for determining the heavy and light chain allotypes of the rabbits used in this study. This work was supported by a grant from the Australian Research Grants Committee.

References

[1] N.Hilschmann and L.C.Craig, Proc. Natl. Acad. Sci. U.S. 53 (1965) 1403.
[2] F.W.Putnam, K.Titani and E.J.Whitley, Proc. Roy. Soc. (London) Ser. B. 166 (1966) 124.
[3] H.D.Niall and P.Edman, Nature 216 (1967) 262.
[4] R.F.Doolittle, Proc. Natl. Acad. Sci. U.S. 55 (1966) 1195.
[5] J.A.Grant, M.E.Lamm and L.Hood, Immunochemistry 6 (1969) 645.
[6] L.Hood, H.Lackland, K.Eichman, T.J.Kindt, D.G.Braun and R.M.Krause, Proc. Natl. Acad. Sci. U.S. 63 (1969) 890.
[7] P.Edman and G.Begg, European J. Biochem. 1 (1967) 80.
[8] M.E.Koshland, F.M.Englberger and S.E.Gaddone, J. Immunol. 89 (1962) 517.
[9] H.B.Levy and H.A.Sober, Proc. Soc. Exptl. Biol. Med. 103 (1960) 250.
[10] J.B.Fleischman, R.H.Pain and R.R.Porter, Arch. Biochem. Biophys. Suppl. 1 (1962) 174.
[11] B.Blomback, M.Blomback, P.Edman and B.Hessel, Biochim. Biophys. Acta 115 (1966) 371.
[12] K.J.Catt, B.Moffat and H.D.Niall, Science 157 (1967) 321.
[13] M.Wallis, FEBS Letters 3 (1969) 118.
[14] A.Teger-Nilsson, Acta Chem. Scand. 22 (1968) 3171.
[15] B.A.Cunningham, P.D.Gottlieb, W.H.Konigsberg and G.M.Edelman, Biochemistry 7 (1968) 1983.
[16] W.A.Schroeder, J.B.Shelton and J.R.Shelton, Arch. Biochem. Biophys. 130 (1968) 551.