# PARTIAL AMINO ACID SEQUENCE IN THE N-TERMINAL REGION OF AN ANTI-*MICROCOCCUS LYSODEIKTICUS* ANTIBODY HEAVY CHAIN OF ALLOTYPE a1

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Received 20 February 1976

## 1. Introduction

Sequence studies of either normal pooled material [1,2] or antibodies of limited heterogeneity [3,4] are in general agreement as to the multiple differences found between the three characterized allotypes (a1, a2 and a3) of the a locus.

The present report describes the partial amino acid sequence of a heavy chain of allotype a1 derived from a homogeneous antibody (designated Ab 120) directed against the carbohydrate of the cell wall of *Micrococcus lysodeikticus*. The sequence for residues 14–16 is different from that reported earlier [1] for IgG Aa1, but agrees with that reported by Johnstone and Mole (manuscript in preparation).

## 2. Materials and methods

Antibodies against *M. lysodeikticus* were induced in rabbits as described earlier [5]. They were characterized by cellulose acetate electrophoresis, precipitin reaction and polyacrylamide gel electrophoresis as reported [5].

Antibody 120 was isolated from a single bleeding and purified from the serum by DEAE cellulose ion exchange chromatography, using a 5 mM sodium phosphate buffer, pH 7.2, as eluent.

Heavy and light chains were separated by gel filtration on Sephadex G 100, in 1 N acetic acid, after mild reduction (0.5 M Tris-HCl, pH 8.2, 0.1 M 2-mercaptoethanol, 1 h at 37°C) and alkylation (0.12 M iodoacetic acid, 30 min at 0°C).

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Cyanogen bromide (CNBr) cleavage of the heavy chain was performed in 70% formic acid for 24 h at 4°C, using a 1:5 weight ratio of protein to CNBr. The N-terminal half of the heavy chain (fragment C-1) from the CNBr digest of the heavy chain, and of the N-terminal 33 residue peptide from fragment C-1 after complete reduction and alkylation with iodo-2-[$^{14}$C] acetic acid, were isolated by gel filtration on G-100 in 6 M urea, 1 N acetic acid, and on G-100 in 1 M $NH_4OH$ respectively. The tryptic digestion of the 33 residue peptide was done using an enzyme:peptide ratio of 1:100 (w/w) in 1% ammonium bicarbonate pH 8.0 at 37°C for a period of 4 h.

Sequence analysis was performed with a Beckman 890 C sequenator using the DMAA program [6]. Identifications of the phenylthiohydantoin derivatives were by gas–liquid chromatography [7], thin-layer chromatography [8] and amino acid analysis on a Durrum 500 D analyser, after back hydrolysis in HI at 150°C for 20 h.

## 3. Results

The complete sequence of the light chain of antibody 120 was determined [9,10] and details will be reported elsewhere.

The PCA (pyrrolidone carboxylic acid)-blocked N-terminal half of heavy chain Ab 120 (fragment C-1) was subjected to 16 automated Edman degradations (fig.1). The homology on comparison with known sequences from rabbit H chains [2,11,12] suggested that fragment C-1 of H chain 120 was cleaved between residues 33 and 34. After complete reduction and alkylation, the blocked N-terminal fragment 1 to 33

was separated from the larger one, residue 34 to 256, by gel filtration. The N-terminal 33 residue peptide of the H-chain was isolated in 65% yield and subjected to tryptic digestion. The whole digest was directly analyzed by automated degradation. The sequence of residue 10–32 was established. Residues 1 to 9 and 33, identified by amino acid analysis of the separated peptides, were placed by homology.

## 4. Discussion

The comparison of the amino acid sequence of the N-terminal 49 residues of H chain of allotypes a1, a2 and a3 is shown in fig.2. Several Aa1 sequences are included in the comparison because of variations in residue 14, 15 and 16. Results by Johnstone and Mole (manuscript in preparation) suggests that the

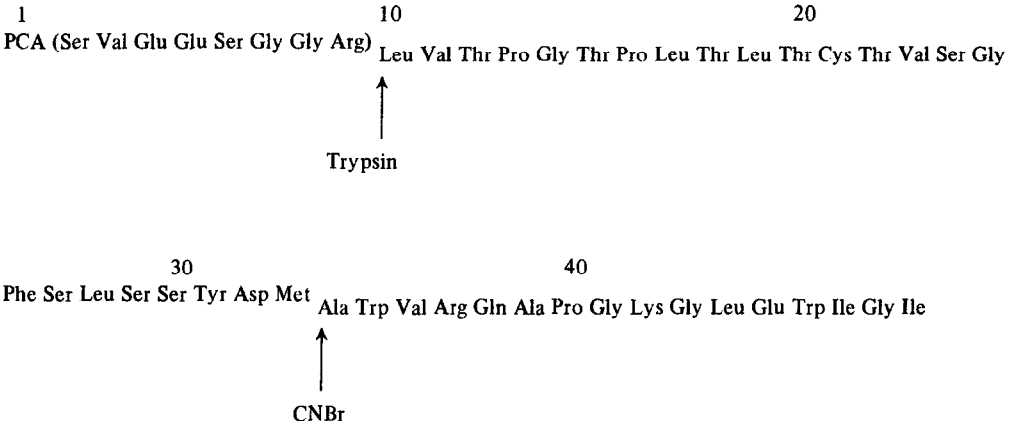


Fig.1. Sequence of fragment 1 to 49 of heavy chain 120. The placing of the peptides is by homology.

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                          10        20        30        40
a1 120 M.L.   O – (S V E E S G G)R L V T P G T P L T L T C T V S G F S L S S Y D M A W V R Q A P G K G L E W I G I
a1 BS5 S 3    O –  S(V E)E S G G R L V T P T P G L T L T C T V S G F S L S S Y D M G W V R Q A P G K G L E W I G I
a1 Pool       O – S V E E S G G R L V T P G T P L T L T C T V S G F S L S S Y A M G W V R Q A P G K G L E W I G I
                  L                       A           D ? S                   Y   F
                                                                                    Y

a2 K25 S 3    O – S V K E S E G G L F K P T D T L T L T C T V S G F S L S G Y D M S W V R Q A P G K G L E W I G V
a2 2690 C     O – S V K E S E G G L F K P T N T L T L T C T V S G I D L S S Y G V S W V R Q A P G K G L E W I G I

a3 Pool       O E S L E E S G G D L V K P G A S L T L T C T A S G F N G S S Y Y M
                  Q       V   ?   ?       V     A   F   ?
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Fig.2. Data for a1 and a3 normal pooled H chains are from Wilkinson [1], Mole et al. [2] and Johnstone and Mole (manuscript in preparation). Data the sequence of anti-(type III pneumococci) H chain BS-5 is from Jaton and Braun [21] and that of anti-(group C streptococcal carbohydrate) H chain R-2690 is from Fleischman [3].

sequence Gly–Thr–Pro which we obtain in Ab 120 heavy chain is also found in normal pooled IgG and IgA preparations of allotype a1. Previous studies using normal pooled IgG concluded in the sequence Thr–Pro–Gly by indirect methods [1].

The only heavy chain (BS-5) which had the sequence Thr–Pro–Gly [12] instead of Gly–Thr–Pro must therefore presumably belong to a rare variant of Aa1 chains not detected in normal pooled IgG or IgA of this allotype.

Previous sequence studies [11] have indicated that homogeneous antibodies may not necessarily contain all the allotypically associated residues of an a locus allotype. Serological analyses have suggested the existence of allotypic variants [13–16]. The complex polymorphism of the a and b genes would lend support to the idea that the various allotypic forms of the a and b locus do not correspond to simple alleles but rather reveal the existence of a regulatory control over coexisting genes [17].

It was on the N-terminal 33 residue portion of fragment C-1 represented in fig.1 that Wilkinson initially correlated the a locus allotype and the a1 and a3 specificities. This study was later completed by Fleischman for the a2 specificity (see fig.2). When using what we believe to be the definitive a1 sequence, with Gly–Thr–Pro instead of Thr–Pro–Gly, the allotype-related sequence correlates may be summarized as:

the residues in positions 9, 15 and 16 are specific for each allotypic form;

the residues in positions 4, 7, 11, 12, 14 and 28 are associated with two of the three forms;

Aa1 and Aa3 sequences are more homologous to each other than to Aa2.

This confirms the serological observations by Brezin and Cazenave [15]. Since the new specificity a100 [18] is closer to allotype a3 than to any other specificity, it may be expected that the sequence will confirm this greater homology.

The parts of the variable regions of the antibody heavy chains represented in fig.2 do not reveal extensive differences, even in the section 30 to 34 which in human myeloma chains corresponds to the first hypervariable region of the heavy chain, possibly involved in antigen binding. Section 35 to 45 is conserved in all the chains, and probably corresponds to the spatial configuration characterized by X-ray diffraction studies on human antibody or myeloma protein fragments [19,20]: two β-pleated sheets joined by a bend at Pro-40 and Gly-41. Studies of the second and third hypervariable regions will usefully complete this initial comparison.

A more precise definition of structure–function relationship clearly makes necessary the determination of a much larger number of antibody heavy chain sequences of similar anti-carbohydrate specificity and of chains from homogeneous antibodies directed against non-carbohydrate antigens.

## Acknowledgements

Mr I. Caplier and U. Lion provided expert technical assistance. The amino acid compositions were kindly determined by Miss M. Van der Linden and Mr Verheulpen.

The authors wish to thank Dr J. D. Capra for the kind assistance in designing the sequenator program, and Drs L. E. Mole and P. A. Cazenave for helpful discussions. The constant support of Dr L. Kanarek is gratefully recognized. (Supported by Government grants 02021 and 02022).

## References

[1] Wilkinson, J. M. (1969) Biochem. J. 112, 173–185.
[2] Mole, L. E., Jackson, S. A., Porter, R. R. and Wilkinson, J. M. (1971) Biochem. J. 124, 301–318.
[3] Fleischman, J. B. (1971) Biochemistry 10, 2753–2761.
[4] Jaton, J. C. (1974) Biochem. J. 143, 723–732.
[5] Van Hoegaerden, M., Wikler, M., Janssens, R. and Kanarek, L. (1975) Eur. J. Biochem. 53, 19–24.
[6] Capra, J. D., Tung, A. S. and Nisonoff, A. (1975) J. Immuol. 115, 414–418.
[7] Pisano, J. J. and Bronzert, T. J. (1969) J. Biol. Chem. 244, 5597–5604.
[8] Summers, M. R., Smythers, G. W. and Oroszlan, S. (1973) Anal. Biochem. 53, 624–628.
[9] Strosberg, A. D. and Van Hoegaerden, M. (1974) Abstr. Proc. Ninth FEBS Meeting, Budapest.
[10] Margolies, M. N., Cannon, L. E., Strosberg, A. D. and Haber, E. (1975) Proc. Nat. Acad. Sci. 72, 2180–2184.
[11] Strosberg, A. D., Jaton, J. C., Capra, J. D. and Haber, E. (1972) Fed. Proc. 31, 771.
[12] Jaton, J. C. and Haimovitch, J. (1974) Biochem. J. 139, 281–283.
[13] Kindt, T. J., Seide, R. K., Tack, B. F. and Todd, C. W. (1973) J. Exp. Med. 138, 33–43.

[14] Oudin, J. (1960) J. Exp. Med. 112, 107–124.
[15] Brezin, C. and Cazenave, P. A. (1975) Immunochem. 12, 241–247.
[16] Knight, K. Personal communication.
[17] Strosberg, A. D. (1976) Trans. Biochem. Soc., in the press.
[18] Cazenave, P. A. and Brezin, C. (1976) Eur. J. Immunol., in the press.
[19] Poljak, R. J., Amzel, L. M., Chen, B. L., Phizackerley, R. P. and Saul, F. (1974) Proc. Nat. Acad. Sci. 71, 3440–3444.
[20] Edmundson, A. B., Ely, K. R., Girling, R. L., Abola, E. E Schiffer, M. and Westholm, F. A. (1974) in: Progress in Immunology II Vol. 1, p. 103, (Brent and Holborow, eds.) North-Holland, Amsterdam.
[21] Jaton, J.-C. and Braun, D. G. (1972) Biochem. J. 130, 539–546.