The N-terminal sequence of albumin Redhill, a variant of human serum albumin

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Received 27 September 1985

Albumin Redhill, a variant human albumin, has been isolated by fast protein liquid chromatofocusing. The N-terminal sequence of this protein corresponded to that of albumin A except that one additional arginine residue was attached to the N-terminus.

Variant albumin *N-terminal sequence* *Arginine* *Chromatofocusing*

1. INTRODUCTION

Bisalbuminemia, the presence in serum of 2 albumins which have different electrophoretic mobilities, is well documented for humans [1]. Whilst many examples of bisalbuminemia have been reported [2], the structures and properties of the variant albumins have been little studied. The amino acid sequence of human albumin A ('normal' albumin) has been determined by conventional sequencing techniques [3] and from the cDNA [4]. In addition, complete amino acid sequences of 6 variant albumins [5–10] and 2 partial sequences [11] have been reported. In 5 of the complete sequences, the mutations in the amino acid chains occur either at the N-terminus [5,8] or near the C-terminus [6,7,9]; only one mutation has been observed in the central region of the protein [10]. The 2 albumins with variant N-termini are both proalbumins and hence each has a hexapeptide attached to the aspartic acid which is the N-terminal amino acid in albumin A. We now wish to report the determination of the N-terminal sequence of albumin Redhill, a variant albumin which we have recently isolated [12].

2. MATERIALS AND METHODS

Mixed albumin A and albumin Redhill was obtained from serum by precipitation with ammonium sulphate followed by chromatography on Sephadex G-100 as described [12]. Pure albumin Redhill was obtained from this mixture by fast protein liquid chromatography (chromatofocusing) using the Pharmacia FPLC system with a monoP HR 5/20 column (Pharmacia, Milton Keynes, England). Aliquots (500 μl) of a solution of the mixed albumins (10–20 mg/ml) in piperazine·HCl buffer (25 mM, pH 6.3) were applied to the monoP column which had already been equilibrated with this buffer. Elution of albumin Redhill was achieved at 1 ml/min with Polybuffer 74 diluted 1:10 (v/v) in water (MilliQ, Millipore, England) which had been brought to pH 4.5 with HCl. The fractions (~1 ml) were desalted on Sephadex G-25 columns (1 × 4 cm) and proteins detected by applying aliquots (10 μl) onto a silica gel TLC plate, drying and spraying with a solution of 1-anilinonaphthalene-8-sulphonic acid (0.003% Mg^{2+} salt in aqueous 0.1 M potassium phosphate, pH 6.8). The presence of protein was indicated by fluorescence when the plate was exposed to radiation at 354 nm. The protein-containing fractions were pooled in 3 peaks, lyophilised and aliquots of the residue suspended in 37% aqueous sucrose (w/v) which contained bromophenol blue (0.01%) for analysis by non-denaturing, discontinuous polyacrylamide gel electrophoresis [13].

The pooled fractions which contained 'pure'

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00145793/85/$3.30

albumin Redhill (>90% pure) were freed of Polybuffer by chromatography on Sephadex G-75 (elution with 0.9% aqueous NaCl (w/v) when the albumin appeared in the first few fractions ahead of the Polybuffer. The protein-containing eluate was dialysed against distilled water and lyophilised. Samples of albumin Redhill (300 μg) were submitted for sequencing by an automated, solid-phase Edman method. The N-terminal sequence of albumin Redhill was Arg-Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu. Our sample of albumin Redhill contained a trace of albumin A and the sequence of this protein was determined at the same time. The N-terminal sequence Asp-Ala-His-Lys-Ser-Glu-Val-Ala-His-Arg-Phe-Lys-Asp-Leu corresponded to that previously published [3,4].

3. RESULTS AND DISCUSSION

Mixed albumin A and albumin Redhill, when applied to the FPLC chromatofocusing column, eluted as 3 main peaks which were (in order of decreasing pH of eluant), albumin A, mixed albumins and albumin Redhill. The N-terminal amino acid of albumin Redhill had previously been shown to be arginine [12] and the partial sequence which we have now determined confirms this observation. Human proalbumin contains 2 arginine residues adjacent to the N-terminal amino acid (aspartic acid) of albumin A [5]. The variant albumins Lille [8] and Christchurch [5] are proalbumins in which one of these arginine residues has been replaced (fig.1) and it has been proposed [8] that mutation of this Arg-Arg sequence prevents cleavage of the N-terminal hexapeptide from proalbumin. In the case of albumin Redhill, cleavage of the proalbumin chain has probably occurred at the N-terminal side of the arginine residue at position −1 (fig.1). If this is so it represents a new type of cleavage of the signal peptide of proalbumin. In this context it is of interest that a sample of bovine serum albumin has been observed which possesses an N-terminal arginine residue [14].

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        -6  -5  -4  -3  -2  -1  1   2   3   4
(a)                                 Asp-Ala-His-Lys
(b)                                 Arg-Asp-Ala-His-Lys
(c)   Arg-Gly-Val-Phe-Arg-Arg-Asp-Ala-His-Lys
(d)   Arg-Gly-Val-Phe-His-Arg-Asp-Ala-His-Lys
(e)   Arg-Gly-Val-Phe-Arg-Gln-Asp-Ala-His-Lys
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Fig.1. N-terminal sequences of (a) albumin A [3,4], (b) albumin Redhill, (c) human proalbumin [5], (d) albumin Lille [8], (e) albumin Christchurch [5].

ACKNOWLEDGEMENTS

We wish to thank Dr R.W. Evans, Guy's Hospital Medical School, London, for assistance with the FPLC purification of albumin Redhill and Dr J.B.C. Findlay, Department of Biochemistry, Leeds University for determining the amino acid sequence.

REFERENCES

[1] Tárnoky, A.L. (1980) Adv. Clin. Chem. 21, 101–146.
[2] Schell, L.M. and Blumberg, B.S. (1977) in: Albumin Structure, Function and Uses (Rosenoer, V.M. et al. eds) pp.113–141, Pergamon, New York.
[3] Meloun, B., Moravek, L. and Kostka, V. (1975) FEBS Lett. 58, 134–137.
[4] Dugaiczyk, A., Law, A. and Dennison, O.E. (1982) Proc. Natl. Acad. Sci. USA 79, 71–75.
[5] Brennan, S.O. and Carrell, R.W. (1978) Nature 274, 908–909.
[6] Winter, W.P., Weitkamp, L.R. and Rucknagel, D.L. (1972) Biochemistry 11, 889–896.
[7] Franklin, S.G., Wolf, S.I., Zweidler, A. and Blumberg, B.S. (1980) Proc. Natl. Acad. Sci. USA 77, 2505–2509.
[8] Abdo, Y., Rousseaux, J. and Dautrevaux, M. (1981) FEBS Lett. 131, 286–288.
[9] Iadarola, P., Minchiotti, L. and Galliano, M. (1985) FEBS Lett. 180, 85–88.
[10] Brennan, S.O. (1985) Biochim. Biophys. Acta 830, 320–324.
[11] Franklin, S.G., Wolf, S.I., Özdemir, Y., Yuregir, G.T., Isbir, T. and Blumberg, B.S. (1980) Proc. Natl. Acad. Sci. USA 77, 5480–5482.
[12] Brand, S., Hutchinson, D.W. and Donaldson, D. (1984) Clin. Chim. Acta 136, 197–202.
[13] Au, H.Y.N., Brand, S., Hutchinson, D.W. and Matejtschuk, P. (1984) IRCS Med. Sci. 12, 56–57.
[14] Leibowitz, M.J. and Soffer, R.L. (1971) J. Biol. Chem. 246, 4431–4438.