Ligand-binding properties of proalbumin Christchurch

Roberta G. REED,*† Theodore PETERS, Jr.,† Stephen O. BRENNAN‡ and Robin W. CARRELL‡

†*The Mary Imogene Bassett Hospital, Cooperstown, NY 13326, U.S.A., and*

‡*Department of Clinical Biochemistry, Christchurch Hospital, Christchurch, New Zealand*

(Received 2 July 1980/Accepted 14 July 1980)

Proalbumin Christchurch, a circulating variant of human serum albumin, is secreted from the liver without cleavage of the hexapeptide situated at the *N*-terminal end of the peptide chain of proalbumin. We compared ligand-binding properties of proalbumin Christchurch and of normal albumin A from the same individual in order to test the effect of the presence of the hexapeptide. The two albumin forms exhibited similar affinities for palmitate, bilirubin, 8-anilidonaphthalene-1-sulphonate and Bromocresol Green. The patterns of endogenous fatty acids bound to the two forms of albumin were slightly different, although the differences were probably not of physiological significance. From these studies it would appear that the propeptide of proalbumin does not alter the protein conformation in such a way as to alter binding sites for organic anions.

A circulating variant of human serum albumin, proalbumin Christchurch, was identified by Brennan & Carrell (1978) in a subject with bisalbuminemia. The variant apparently differs from normal albumin (albumin A) only by the presence of an additional hexapeptide segment, Arg-Gly-Val-Phe-Arg-Gln, at the *N*-terminal end of the chain. The glutamine residue in the *C*-terminal position replaces the arginine found in the propeptides of rat proalbumin and bovine proalbumin (Russell & Geller, 1975; Patterson & Geller, 1977); thus proalbumin Christchurch lacks a double basic amino acid sequence and is not cleaved before secretion from the liver cell.

The function of the propeptide of proalbumin is not known, but among the suggested roles is that it blocks a binding site for a physiologically important ligand while the albumin is within the liver cell. The proteolytic activity of trypsinogen is blocked in just such a manner by an *N*-terminal peptide segment (Walsh & Neurath, 1964). We now report tests of the binding of two important natural ligands, palmitate and bilirubin, and of two additional organic anions to proalbumin Christchurch and to albumin A prepared from a heterozygous individual.

Materials

Proalbumin Christchurch and albumin A were isolated from the serum of a patient with bisalbuminemia as described previously (Brennan &

Carrell, 1978). A commercial preparation from pooled human plasma was tested for comparison; this was a human albumin fraction V preparation that had been protected against dimerization by blocking the thiol group with half-cystine followed by deionization (from Hyland Diagnostics, Deerfield, IL, U.S.A.).

Methods

Palmitate binding was determined by partition of [14 C]palmitate between heptane and the protein in 0.1 M-NaCl/20 mM-sodium phosphate buffer, pH 7.4, at 37°C (Reed *et al.*, 1975a). Bilirubin binding was measured in the same buffer at 24°C by equilibration with bovine albumin-agarose (Reed *et al.*, 1975b). Spectral changes on binding of bilirubin were measured in an Aminco DW-2A spectrophotometer at 24°C; an appropriate amount of bilirubin freshly dissolved in 10 mM-NaOH containing 1 mM-EDTA was added to a 15 μ M solution of albumin in the saline/phosphate buffer, pH 7.4. Binding of 8-anilidonaphthalene-1-sulphonate and Bromocresol Green was assayed by measuring fluorescence and A_{630} respectively, as described previously (Reed *et al.*, 1975a). Fatty acid content of the albumins was determined by using a Perkin-Elmer Sigma 3 gas chromatograph with a 3.2 mm $\times$ 1.83 m ($\frac{1}{8}$ in $\times$ 6 ft) 5% DEGS (diethylene glycol succinate) column at 175°C with a flame ionization detector. The fatty acids were first extracted from an acidified aqueous solution of the albumins with heptane and esterified with BF₃/methanol. Precision of the assay was about 5%.

* To whom reprint requests should be addressed.

Protein concentration was determined by the biuret reaction, with crystalline bovine albumin as standard, on the basis of an a_{280} value for bovine albumin of $0.661 \text{ litre} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ (Doumas, 1975). The a_{280} values for proalbumin Christchurch and albumin A were found to be 0.547 and 0.532 $\text{litre} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ respectively.

Results

Binding of palmitate by defatted proalbumin Christchurch and albumin A is shown in Fig. 1(a) as a Scatchard plot. The interaction of long-chain fatty acids with albumin is most successfully described by a series of sites with decreasing affinity. As many as nine different sites may contribute to the binding profile (Spector, 1975). The identity of the data for albumin and proalbumin Christchurch indicates that the principal binding sites of albumin must be the

same on albumin A and proalbumin Christchurch to provide the composite binding curve observed.

The optical absorption spectra of bound bilirubin were nearly identical for the two albumin forms over the range 350–550 nm at molar ratios of bilirubin to albumin of 0.45:1.0 and 0.90:1.0. The absorbance maximum was at 458 nm for proalbumin Christchurch, 457 nm for albumin A and 460 nm for the control albumin from pooled plasma. Molar absorption coefficients of the bound bilirubin were 40 100, 40 200 and 42 000 $\text{litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ respectively for the three albumins.

Affinity for bilirubin exhibited by proalbumin Christchurch was the same as that exhibited by albumin A (Fig. 1b) and a commercial albumin pool (not shown). The Scatchard plot is consistent with one strong site and one or two somewhat weaker sites, as has been reported for human albumin (Broderson, 1979).

Yield of colour with Bromocresol Green, probably the most widely used reagent for assay of albumin in serum, was similar with the two albumin species from our subject and with the control albumin. The average a_{630} was $2.72 \text{ litre} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$ for the three preparations over an albumin concentration range 25–200 mg/ml. Fluorescence observed with 8-anilidonaphthalene-1-sulphonate, another ligand with high specificity for albumin, was identical for the two albumin forms over an albumin concentration range 7–18 mg/ml, although about 20% lower than the fluorescence with the control preparation. The difference probably reflects the different methods of isolation for control and sample albumins.

Table 1 shows the relative composition of the fatty acids extractable from the albumins with heptane. Albumin A and the variant form both contained more myristate and stearate and less oleate and linoleate than did the pooled control albumin. On comparison of the two albumin forms isolated from the same subject, proalbumin Christchurch carried less myristate and more stearate than did albumin A.

Fig. 1. Scatchard plots of binding of (a) palmitate and (b) bilirubin to proalbumin Christchurch and albumin A. Binding of palmitate was determined by partition in heptane of [^{14}C]palmitate at pH 7.4 and 37°C . Binding of bilirubin was determined by competition with bovine albumin–agarose at pH 7.4 and 24°C . ●, Proalbumin Christchurch; ○, albumin A. $\bar{v}$ is the molar ratio of bound ligand to albumin; c is the concentration of free ligand.

Table 1. Relative fatty acid contents of albumin preparations

Fatty acid	Relative fatty acid content (%)		
	Proalbumin Christchurch	Albumin A	Pooled albumin
C _{14:0}	18	31	4
C _{16:0}	30	26	23
C _{16:1}	4	7	5
C _{18:0}	22	11	9
C _{18:1}	7	8	30
C _{18:2}	1	2	13
C _{18:3}	2	1	2
C _{>18}	16	14	14

Discussion

The present studies show that proalbumin Christchurch binds palmitate, bilirubin and two indicator compounds specific for albumin equally as well as does the normal albumin A isolated from the serum of the same individual. In a separate study, proalbumin isolated from rat liver was found to bind palmitate and bilirubin as well as did rat serum albumin (Peters & Reed, 1980). Hence the function of the additional hexapeptide in proalbumin will have to be sought elsewhere than in suppression of binding of these ligands.

The differences in fatty acid composition (Table 1) between proalbumin Christchurch and albumin A may point to slight differences in relative affinity for different fatty acid species, the variant binding stearate more strongly and myristate less strongly than does the normal form, although both bind palmitate to the same extent. However, the difference is not striking and is probably not of physiological import. The increase in myristate and decrease in stearate in the two albumin forms obtained in New Zealand relative to the control albumin from pooled serum obtained in the U.S.A. would seem to be the result of dietary differences.

One functional defect of proalbumin has been observed. Proalbumin Christchurch and rat proalbumin both fail to bind copper(II) or nickel(II) (Brennan & Carrell, 1979; Peters & Reed, 1980). This defect was predictable, because the copper-

binding site is known to lie at the *N*-terminus of these albumins and to include the α -amino nitrogen, a site that is blocked in proalbumin. The physiological consequence of this defect is not known.

This work was supported by U.S. Public Health Service Research Grant HL-02751, the Stephen C. Clark Research Fund of The Mary Imogene Bassett Hospital and the New Zealand Medical Research Council. We are indebted to Mr. James Welty for technical assistance.

References

- Brennan, S. O. & Carrell, R. W. (1978) *Nature (London)* **274**, 908–909
- Brennan, S. O. & Carrell, R. W. (1979) *Biochim. Biophys. Acta* **621**, 83–88
- Broderson, R. (1979) *J. Biol. Chem.* **254**, 2364–2369
- Doumas, B. T. (1975) *Clin. Chem.* **21**, 1159–1166
- Patterson, J. E. & Geller, D. M. (1977) *Biochem. Biophys. Res. Commun.* **74**, 1220–1226
- Peters, T., Jr. & Reed, R. G. (1980) *J. Biol. Chem.* **255**, 3156–3163
- Reed, R. G., Feldhoff, R. C., Clute, O. L. & Peters, T., Jr. (1975a) *Biochemistry* **14**, 4578–4583
- Reed, R. G., Gates, T. & Peters, T., Jr. (1975b) *Anal. Biochem.* **69**, 361–371
- Russell, J. H. & Geller, D. M. (1975) *J. Biol. Chem.* **250**, 3409–3414
- Spector, A. A. (1975) *J. Lipid Res.* **16**, 165–179
- Walsh, K. & Neurath, H. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **52**, 884–889