J. gen. Virol. (1973), **19**, 391–395
Printed in Great Britain

Affinity Chromatography of Hepatitis B Antigen on Concanavalin A Linked to Sepharose

(*Accepted* 9 *February* 1973)

Concanavalin A (Con A), a lectin isolated from jack beans, binds specifically to saccharides with terminal α-D-mannopyranosyl, α-D-glucopyranosyl or β-D-fructofuranosyl residues (Goldstein, Hollerman & Merrick, 1965*a*; Goldstein, Hollerman & Smith, 1965*b*; Goldstein & So, 1965). Glycoproteins, present in human serum (Leon, 1967; Morse, 1968) or representing structural components of membranes of mammalian cells (Inbar & Sachs, 1969) or of enveloped viruses (Oram *et al.* 1971; Becht, Rott & Klenk, 1972; Calafat & Hageman, 1972; Klenk, Rott & Becht, 1972) react with Con A. Recently, Cawley (1972) observed that Con A partially precipitated hepatitis B antigen. Results presented here extend this finding and show that the interaction between hepatitis B antigen and Con A may be utilized as a step in purifying the distinct morphological forms of this antigen.

Four vol. of serum containing hepatitis B antigen were mixed with increasing amounts (0·2 to 1·0 vol.) of a solution of Con A (1 g in 26·4 ml of saturated NaCl; Miles-Yeda Limited, Rehovoth, Israel). The mixtures were kept overnight at 4 °C and then centrifuged at 15000 rev/min for 20 min. The pellets were redissolved in 5 % (w/v) methyl-α-D-mannopyranoside (MMP; Calbiochem, La Jolla, California) in 0·02 M-tris-maleate, pH 6·0. Hepatitis B antigen was determined in the supernatant fluids and in the redissolved pellets by immunoelectro-osmophoresis (Prince & Burke, 1970). The relative concentration of the antigen was expressed as the reciprocal of the highest dilution of the sample at which a precipitin line was still observed. Tris-maleate buffer (0.02 M) (TM) containing 5 % (w/v) MMP was used to dilute the samples. The relative concentration of antigen in the original serum was estimated in the same way. Hepatitis B antigen was absent from the supernatant fluids only when the highest level (1 part for 4 parts of serum) of Con A was used. All the antigen was recovered in the redissolved pellets, the vol. of which was reduced 5 to 10 times as compared to the vol. of serum.

Both common (*a*) and mutually exclusive subtype specific (*d* or *y*) antigenic determinants are exposed on the surface of hepatitis B antigen particles (Le Bouvier, 1971). Both *ad* and *ay* subtypes of the antigen were precipitated with Con A.

In addition to hepatitis B antigen, several serum proteins, mainly IgM, IgA, α_1-antitrypsin, α_2-macroglobulin, haptoglobin, ceruloplasmin and components of complement, are precipitated with Con A (Leon, 1967). However, about 85 % of serum proteins were not precipitated. The glucose present in serum (about 4·3 g/l) due to collection of blood into a citric acid–glucose solution, failed to prevent the precipitation. Glucose inhibited the precipitation of dextrans with Con A, albeit approximately 40 times less efficiently than MMP (Goldstein *et al.* 1965*b*). The minimal amount of Con A required for complete precipitation of the antigen was not diminished when glucose was removed from the serum by dialysis.

To isolate hepatitis B antigen from the redissolved precipitate, a method was required for separating antigen from Con A. Neither rate zonal sedimentation nor molecular exclusion chromatography was suitable for this purpose because of the formation of aggregates of Con A containing fragments of the subunit of Con A (Wang, Cunningham & Edelman,

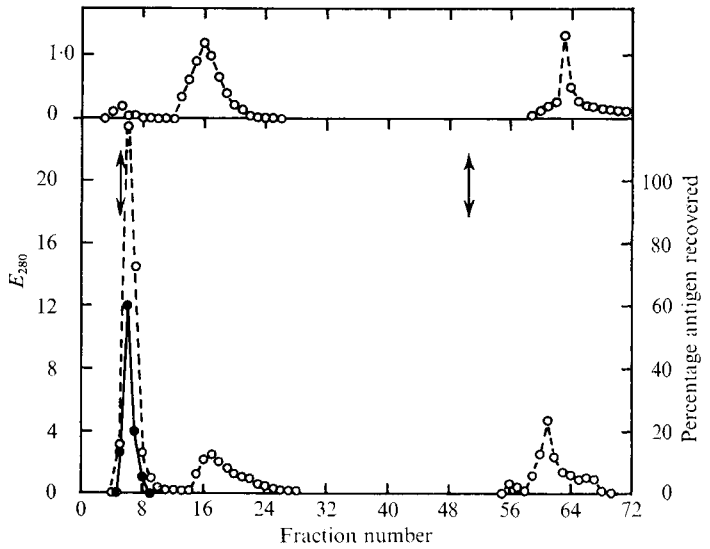

Fig. 1. Chromatography of a mixture of hepatitis B antigen, Con A and Con A-binding serum proteins (bottom) and of Con A (top) on SP-Sephadex. O – – O, E_{280}; ●—●, percentage antigen recovered. Left and right arrows indicate the start of elution by a linear gradient of increasing NaCl concentration (0 to 0·6 M) and by 0·2 M-NaOH, respectively. Hepatitis B antigen and the major part of serum proteins were recovered in fractions corresponding to the void vol. of the column. The extinction results were derived from measurements on 15-fold dilutions of fractions.

1971; Cunningham *et al.* 1972). Con A consists of several species differing in isoelectric points (pI) and in the presence of sugars binding to Con A, the pI are shifted to higher values between pH 7·8 and 8·0 (Akedo *et al.* 1972). Hepatitis B antigen appears also to consist of species with distinct pI between pH 3·6 and 4·6 (C. R. Howard & A. J. Zuckerman, personal communication; Dreesman *et al.* 1972). It was expected that chromatography on ion-exchange resins would be best suited for separating hepatitis B antigen from Con A since their pI differ considerably.

One ml of a suspension of partially purified hepatitis B antigen, obtained from serum by precipitation with Con A and subsequent dissolution of the precipitate in 5% MMP in TM, was applied on top of a 22 × 1 cm column of sulphopropyl (SP)-Sephadex C-25 (Pharmacia, Uppsala, Sweden). The column was washed with 4 ml of 5% MMP in TM. A continuous linear gradient of increasing NaCl concentration (25 ml 5% MMP in TM and 25 ml 0·6 M-NaCl in 5% MMP in TM) was used for elution, which was completed with 0·2 M-NaOH. Fractions of 1 ml each were collected. One ml of a 3·9% (w/v) solution of Con A, which had been dialysed against 5% MMP in TM, was chromatographed under the same conditions. The results shown in Fig. 1 indicate that hepatitis B antigen and Con A were separated by chromatography on SP-Sephadex.

The fractionation of serum proteins was also achieved by affinity chromatography on Con A covalently linked to Sepharose (Con A Sepharose), with results similar to those obtained by precipitation with soluble Con A (Aspberg & Porath, 1970). In order to avoid the separation of hepatitis B antigen and Con A, the purification of the antigen by affinity chromatography was investigated. One ml of antigen-positive serum was applied on top of a 17 × 1 cm column of Con A Sepharose (Pharmacia, Uppsala, Sweden), prewashed with 0·14 M-NaCl, 0·01 M-tris, 0·001 M-$CaCl_2$, 0·001 M-$MnCl_2$ pH 7·2 (TCaMn). Subsequently the

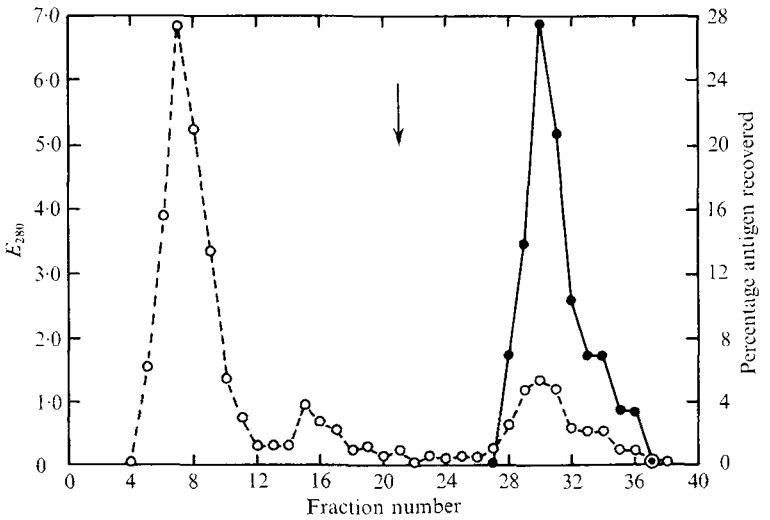

Fig. 2. Affinity chromatography of hepatitis B antigen on Con A Sepharose. ○——○, E_{280}; ●——●, percentage antigen recovered. Arrow indicates the start of elution by 5% MMP in TM. The extinction of fractions was determined as described for Fig. 1.

column was washed with 20 ml of TCaMn, followed by 5% MMP in 0·14 M-NaCl, 0·01 M-tris pH 7·2 to elute hepatitis B antigen. Fractions of 1·2 ml each were collected. The results shown in Fig. 2 indicate that approximately 82% of serum proteins were removed from the antigen by affinity chromatography. Subsequent experiments showed that the maximal vol. of serum which could be chromatographed under conditions allowing complete retention of hepatitis B antigen corresponded to approximately 24% of the vol. of Con A Sepharose. The adsorbent could be reused and no losses of activity were detected within 3 months.

Hepatitis B antigen, partially purified by either of the procedures described before, was further purified by molecular exclusion chromatography on a 56 × 0·9 cm column of controlled-pore glass beads (498 Å pore size, 120 to 200 mesh; Electro Nucleonics, Incorporated Fairfield, New Jersey) coated with polyethylene glycol 20000 (Hawk, Cameron & Dufault, 1972) and subsequently with haemoglobin (authors' unpublished data). Fractions corresponding to the void vol. of the column, which contained hepatitis B antigen, were pooled, concentrated to 1 ml by ultrafiltration, layered on top of 1 ml of 25% (w/v) sucrose in 0·14 M-NaCl, 0·01 M-tris pH 72, and centrifuged for 3 h at 50000 rev/min in the Spinco rotor SW 65. The pellet, resuspended in 0·025 M-phosphate pH 7·2, was negatively stained with 1% phosphotungstate and examined by electron microscopy. Spherical particles of approximately 40 and 22 nm diameter and rods 22 nm wide were observed (Fig. 3). Thus all three morphological forms of hepatitis B antigen, which share common surface antigens (Dane, Cameron & Briggs, 1970; Gust *et al.* 1970; Jokelainen *et al.* 1970; Cossart *et al.* 1971) have Con A binding sites exposed on their surface.

The methods described here may contribute to the purification and separation of different forms of hepatitis B antigen with preserved biological activity, and may facilitate the characterization of the causative agent of serum hepatitis. Previously published procedures for the purification of the smaller spherical particles of hepatitis B antigen in which proteolytic enzymes (Millman *et al.* 1970) or an environment of low or high pH (De Rizzo *et al.* 1972) were used, may be deleterious to the other morphological forms of the antigen. The

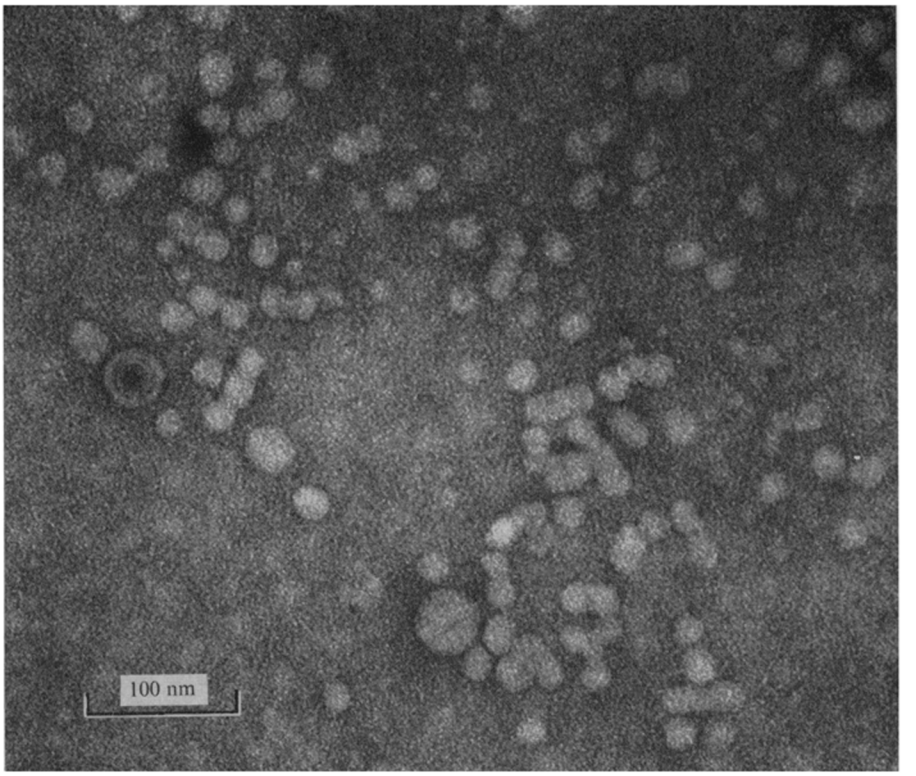

Fig. 3. Particles of hepatitis B antigen adsorbed to and subsequently eluted from Con A Sepharose.

larger spherical particles and the filamentous forms of the antigen were disrupted in the method used by Dreesman *et al.* (1972). The 40 nm particles tended to break down rapidly following isolation by isopycnic banding in CsCl gradients and rate zonal sedimentation (Hall, 1972).

Affinity chromatography on Con A Sepharose may be generally useful for the purification of enveloped viruses with Con A binding sites.

This study was supported by grants HE-09011 and AI-09516 from the National Institutes of Health and by Contract 70-2236 from the National Heart and Lung Institute. A. M. Prince is a Career Scientist of the Health Research Council of the City of New York under Contract I-533.

Virology Laboratory
The New York Blood Center
New York, New York 10021
U.S.A.

A. R. NEURATH
A. M. PRINCE
A. LIPPIN

REFERENCES

AKEDO, H., MORI, Y., KOBAYASHI, M. & OKADA, M. (1972). Changes of isoelectric points of Concanavalin A induced by the binding of carbohydrates. *Biochemical and Biophysical Research Communications* **49**, 107–113.

ASPBERG, K. & PORATH, J. (1970). Group-specific adsorption of glycoproteins. *Acta chemica scandinavica* **24**, 1839–1841.

BECHT, H., ROTT, R. & KLENK, H.-D. (1972). Effect of Concanavalin A on cells infected with enveloped RNA viruses. *Journal of General Virology* **14**, 1–8.

CALAFAT, J. & HAGEMAN, P. C. (1972). Binding of Concanavalin A to the envelope of two murine RNA tumour viruses. *Journal of General Virology* **14**, 103–106.

CAWLEY, L. P. (1972). Reaction between Concanavalin A and the Australia antigen. *American Journal of Clinical Pathology* **57**, 253.

COSSART, Y. E., FIELD, A. M., HARGREAVES, F. D. & PORTER, A. A. (1971). Morphology and immunological properties of Australia/SH antigen in hepatitis. *Microbios* **3**, 5–14.

CUNNINGHAM, B. A., WANG, J. L., PFLUMM, M. N. & EDELMAN, G. M. (1972). Isolation and proteolytic cleavage of the intact subunit of Concanavalin A. *Biochemistry* **11**, 3233–3239.

DANE, D. S., CAMERON, C. H. & BRIGGS, M. (1970). Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet* i, 695–698.

DE RIZZO, E., PANDEY, R., WALLIS, C. & MELNICK, J. L. (1972). Concentration and purification of hepatitis B antigen with polyethylene glycol and polyelectrolyte 60, a cross-linked copolymer of isobutylene maleic anhydride. *Infection and Immunity* **6**, 335–338.

DREESMAN, G. R., HOLLINGER, F. B., SURIANO, J. R., FUJIOKA, R. S., BRUNSCHWIG, J. P. & MELNICK, J. L. (1972). Biophysical and biochemical heterogeneity of purified Hepatitis B antigen. *Journal of Virology* **10**, 469–476.

GOLDSTEIN, I. J., HOLLERMAN, C. E. & MERRICK, J. M. (1965*a*). Protein-carbohydrate interaction. I. The interaction of polysaccharides with Concanavalin A. *Biochimica et biophysica acta* **97**, 68–76.

GOLDSTEIN, I. J., HOLLERMAN, C. E. & SMITH, E. E. (1965*b*). Protein–carbohydrate interaction. II. Inhibition studies on the interaction of Concanavalin A with polysaccharides. *Biochemistry* **4**, 876–883.

GOLDSTEIN, I. J. & SO, L. (1965). Protein-carbohydrate interaction. III. Agar gel-diffusion studies on the interaction of Concanavalin A, a lectin isolated from jack bean, with polysaccharides. *Archives of Biochemistry and Biophysics* **111**, 407–414.

GUST, I. D., CROSS, G., KALDOR, J. & FERRIS, A. A. (1970). Virus-like particles in Australia-antigen-associated hepatitis. *Lancet* i, 953.

HALL, W. T. (1972). Ultrastructural features of Australia antigen. In *Hepatitis and Blood Transfusion*, pp. 227–233. Edited by G. N. Vyas, H. A. Perkins and R. Schmid. New York and London: Grune and Stratton.

HAWK, G. L., CAMERON, J. A. & DUFAULT, L. B. (1972). Chromatography of biological materials on polyethylene glycol treated controlled-pore glass. *Preparative Biochemistry* **2**, 193–202.

INBAR, M. & SACHS, L. (1969). Interaction of the carbohydrate-binding protein Concanavalin A with normal and transformed cells. *Proceedings of the National Academy of Sciences of the United States of America* **63**, 1418–1425.

JOKELAINEN, P. T., KROHN, K., PRINCE, A. M. & FINLAYSON, N. D. C. (1970). Electron microscopic observations on virus-like particles associated with SH antigen. *Journal of Virology* **6**, 685–689.

KLENK, H.-D., ROTT, R. & BECHT, H. (1972). On the structure of the influenza virus envelope. *Virology* **47**, 579–591.

LE BOUVIER, G. L. (1971). The heterogeneity of Australia antigen. *Journal of Infectious Diseases* **123**, 671–675.

LEON, M. A. (1967). Concanavalin A reaction with human normal immunoglobulin G and myeloma immunoglobulin G. *Science* **158**, 1325–1326.

MILLMAN, I., LOEB, L. A., BAYER, M. E. & BLUMBERG, B. S. (1970). Australia antigen (a hepatitis-associated antigen). Purification and physical properties. *Journal of Experimental Medicine* **131**, 1190–1199.

MORSE, J. H. (1968). Immunological studies of phytohaemagglutinin. I. Reaction between phytohaemagglutinin and normal sera. *Immunology* **14**, 713–724.

ORAM, J. D., ELLWOOD, D. C., APPLEYARD, G. & STANLEY, J. L. (1971). Agglutination of an arborvirus by Concanavalin A. *Nature New Biology* **233**, 50–51.

PRINCE, A. M. & BURKE, K. (1970). Serum hepatitis antigen (SH): Rapid detection by high voltage immuno-electro-osmophoresis. *Science, New York* **169**, 593–595.

WANG, J. L., CUNNINGHAM, B. A. & EDELMAN, G. M. (1971). Unusual fragments in the subunit structure of Concanavalin A. *Proceedings of the National Academy of Sciences of the United States of America* **68**, 1130–1134.

(*Received* 1 *January* 1973)