The Fractionation and Isolation of DNA-Binding Proteins from Human Serum

ANWAR A. SIDDIQUI, R. JEREMY H. DAVIES and J. ALAN HILL

Department of Biochemistry, Medical Biology Centre, The Queen's University of Belfast, Belfast BT9 7BL, N. Ireland, U.K.

Human blood serum contains a number of anionic DNA-binding proteins, which can be isolated from it by chromatography on DNA-cellulose. It has been estimated that the DNA-binding proteins amount to 1.5% of total serum protein and, as judged by SDS-gel electrophoresis, they comprise at least 20 different species of protein subunits (Brehm *et al.*, 1975). At present, the identity and physiological function of most of these proteins remain unknown. Krøll *et al.* (1976) have used specific antisera to detect the presence of some known serum proteins in the DNA-binding proteins derived from human plasma. Hoch & McVey (1977) have purified and characterized two of the major serum DNA-binding proteins, denoted DNA-binding protein 1 (DBP1) and DNA-binding protein 2 (DBP2); both are monomeric glycoproteins with respective mol.wts. of 126000 and 86000. The greatest interest in the individual serum DNA-binding proteins has, however, centred on a fragment of complement component C3 (with subunits of mol.wt. 74000, 40000 and 22000) which is present at elevated concentrations in foetal cord serum and sera from patients with a wide range of malignant diseases (Parsons & Hoch, 1976). This protein, designated C3DP, shows considerable promise as a diagnostic and prognostic indicator in these conditions (Parsons *et al.*, 1977, 1978). More recently, malignant disease has been correlated with the absence of a particular DNA-binding protein which is present in normal serum (Lewis & André, 1978).

To achieve a better understanding of the structure and functions of the various serum DNA-binding proteins we are attempting to fractionate and characterize them systematically with chromatographic and electrophoretic techniques. In this communication we describe a chromatographic fractionation procedure which has led to the isolation of three species of serum DNA-binding proteins, which do not appear to have been investigated hitherto. The fractionation procedure is based on the original method used by Brehm *et al.* (1975) to obtain DNA-binding proteins from human serum but incorporates certain modifications. The protein composition of chromatographic fractions was routinely assayed by analytical SDS-slab gel electrophoresis after reduction with 2-mercaptoethanol (Laemmli, 1970).

Pooled normal human serum (200ml) was loaded on to a QAE-Sephadex column (4.5cm × 50cm) equilibrated in 10mM-potassium phosphate buffer, pH6.5 (containing 1mM-2-mercaptoethanol, 1mM-EDTA and 0.1mM-phenylmethylsulphonyl fluoride). The column was washed with this buffer until all unbound material was removed. A linear gradient of 0–500mM-NaCl in the pH6.5 buffer (total volume 1600ml) was then applied. Absorbance measurements at 280nm indicated that the major protein peaks were eluted from the column as the NaCl concentration in the eluate increased from 75 to 175mM, with smaller amounts of protein eluted at higher NaCl concentrations. As the peaks were not clearly resolved the column eluate was pooled to give four fractions corresponding to NaCl concentrations of 75–100mM, 110–120mM, 125–175mM and 180–225mM. Electrophoretic analysis showed each of these fractions to contain a distinct and reproducible spectrum of protein components. In this work the DNA-binding proteins eluted in the 125–175mM-NaCl fraction were investigated.

After dialysis this fraction was rechromatographed on a smaller QAE-Sephadex column (4.5cm × 20cm) under the same conditions. After washing with pH6.5 buffer containing 75mM-NaCl, two protein fractions were collected by stepwise elution, at first 150mM-NaCl and then 200mM-NaCl. The major DNA-binding proteins found in the first of these fractions corresponded to DBP1 and DBP2 (Hoch & McVey, 1977). The major DNA-binding proteins in the second fraction were characterized as follows.

The fraction was dialysed against 10mM-potassium phosphate buffer, pH6.8, loaded

on to a column (15cm×2cm) of DNA-cellulose (Litman, 1968) and then washed with the same buffer containing 50mM-NaCl. A linear gradient of 50–500mM-NaCl was then applied. Electrophoretic analysis of the eluate showed that there were three predominant DNA-binding protein species, which eluted together over the range 90–120mM-NaCl. Fractions containing these proteins were combined, concentrated and then applied to a gel-filtration column of Sephadex G-200 (2cm×90cm) in 100mM-potassium phosphate buffer, pH7.0, containing 500mM-NaCl. The elution profile showed one absorbance peak corresponding to the void volume of the column followed by a second well-defined peak.

The material in the void volume peak proved to be a glycoprotein whose native mol.wt. was estimated to be in excess of 500000 from its mobility on gradient-pore gel electrophoresis. After reduction, it gave one band on SDS-gel electrophoresis corresponding to a subunit mol.wt. of 72000. Isoelectric focusing showed a major component with pI6.4. Minor bands with pI6.2 and 6.1 may represent trace contaminants or indicate that the glycoprotein is polymorphic.

The second peak from the gel-filtration column contained two DNA-binding proteins whose mol.wts. were estimated from SDS-polyacrylamide gels as 68000 and 100000. Both appear to be monomeric, as judged by their electrophoretic mobilities under non-denaturing conditions. There is a definite tendency for these species to associate with one another, which has hampered attempts to isolate them individually. However, a partial purification of each component has been achieved.

Brehm, S. P., Hoch, S. O. & Hoch, J. A. (1975) *Biochem. Biophys. Res. Commun.* **63**, 24–31
Hoch, S. O. & McVey, E. (1977) *J. Biol. Chem.* **252**, 1881–1887
Krøll, J., Larsen, J. K., Loft, H., Ezban, M., Wallevik, K. & Faber, M. (1976) *Biochim. Biophys. Acta* **434**, 490–501
Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
Lewis, J. G. & André, C. M. (1978) *FEBS Lett.* **92**, 211–213
Litman, R. M. (1968) *J. Biol. Chem.* **243**, 6222–6233
Parsons, R. G. & Hoch, J. A. (1976) *Eur. J. Biochem.* **71**, 1–8
Parsons, R. G., Longmire, R. L., Hoch, S. O. & Hoch, J. A. (1977) *Cancer Res.* **37**, 692–695
Parsons, R. G., Hoch, J. A. & Longmire, R. L. (1978) *Cancer* **41**, 2099–2106

The Two-Dimensional Electrophoresis of Human Serum DNA-Binding Proteins

ANNE E. HUGHES,* W. SIDNEY B. LOWRY,* J. ALAN HILL† and R. JEREMY H. DAVIES†

**Department of Oncology and † Department of Biochemistry, The Queen's University of Belfast, Belfast BT9 7BL, N. Ireland, U.K.*

DNA-binding proteins are a class of anionic serum proteins which are isolated by their ability to bind to DNA-cellulose. At least 20 components have been resolved by polyacrylamide-gel electrophoresis in the presence of SDS (Brehm *et al.*, 1975). Up to 18 proteins have been detected by crossed immunoelectrophoresis (Krøll *et al.*, 1976). The significance of their DNA-binding property is unknown. It has been suggested that some of these proteins may be involved in the regulation of gene expression (Kubinski & Javid, 1973), although none of those identified so far (Krøll *et al.*, 1976) appears to function in this way.

Two major proteins, designated DNA-binding protein 1 and DNA-binding protein 2 (Hoch & McVey, 1977), together with C3DP, a fragment of complement component C3 (Parsons & Hoch, 1976), have been purified and partially characterized. The concentrations of C3DP (Parsons *et al.*, 1977) and of another minor DNA-binding protein (Lewis & André, 1978) are altered in serum samples obtained from foetal cord and from patients with malignant diseases.