DNA-BINDING PROTEINS IN HUMAN SERUM

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SUMMARY: Human serum DNA-binding proteins were isolated by affinity chromatography on DNA-cellulose. These proteins represent a significant fraction (1.5%) of the serum proteins. The isolated proteins were quantitatively assayed by a nitrocellulose membrane filter technique using human DNA. Analysis by radioimmunoelectrophoresis revealed that the major DNA-binding proteins migrated in the α_2 and β regions. Using thirty-nine different antisera against major serum proteins, Ouchterlony double diffusion analysis gave positive precipitin reactions only with antisera against immunoglobulin G and α_1-antichymotrypsin. Neither of these comprised a major fraction of the DNA-binding proteins. Chromatography through Sephadex G-150 indicated that the proteins ranged in molecular weight from 50,000 to greater than 400,000. Sodium dodecyl sulfate gel electrophoresis revealed two major protein bands with molecular weights of 126,000 and 94,000, and greater than 20 minor species.

DNA-binding proteins have been demonstrated in mammalian physiological fluids such as human cerebrospinal fluid (1) and the serum of animal species which include dog, mink, horse, sheep, cow and cat (2). In the latter study, Thoburn et al. purified to homogeneity an anionic DNA-binding protein from canine sera. They were unable to demonstrate the presence of such a protein in normal human serum using the methods of hemagglutination and precipitation. However, using a membrane filter assay, Kubinski and Javid previously had reported DNA-binding proteins in human serum (3). This communication describes the first specific isolation and partial characterization of the DNA-binding proteins from human serum.

MATERIALS AND METHODS

DNA-cellulose - The method of Litman (4) was followed in preparing DNA-cellulose, using acid-washed cellulose (Whatman CF-11) and native calf thymus DNA (Sigma, Type V). The yield averaged 12 mg of DNA bound per g of cellulose.

Electrophoresis - SDS slab gel electrophoresis was carried out using the system of Laemmli (5). The apparatus was patterned on that of Studier (6). After electrophoresis, the slabs were stained using the procedure of Fairbanks et al. (7).

Abbreviations: SDS, sodium dodecyl sulfate; IgG, immunoglobulin G

DNA-binding protein assay - The assay system used was a modification of the procedures of Yarus and Berg (8) and Bourgeois (9). The reaction mixture contained in a volume of 0.5 ml: sodium borate buffer, pH 7.0, 40 µmoles; bovine serum albumin, 10 µg; [^{14}C]Wil$_2$ DNA, 2.5 µg; and protein to be assayed. The samples were then slowly filtered on a manifold filtering apparatus through membrane filters (Schleicher and Schuell, B6, 25 mm), washed 3 times with 1.5 ml aliquots of the same buffer, dried and counted. Controls containing the reaction mixture minus DNA-binding protein retained less than 1% of the original total counts. Protein was measured by the method of Lowry et al. (10) using bovine serum albumin as standard. A human, diploid lymphocyte cell line, Wil$_2$, was the source of the assay DNA.

Labeling of DNA-binding proteins with ^{125}I - Proteins from the QAE-Sephadex II fraction (120 µg) were labeled with ^{125}I (0.27 mCi, New England Nuclear) by the method of David (11) using Sepharose-coupled bovine lactoperoxidase. The reaction mixture was passed through a column of 0.5 ml IR-45 under 4 ml Sephadex G-25, prewashed with 0.1% bovine serum albumin, to remove unreacted isotope. The protein was eluted with phosphate buffered saline plus 0.025 M NaN_3.

RESULTS

Extraction of DNA-binding proteins - The DNA-binding proteins in human serum were isolated from the bulk of the serum components by affinity chromatography on DNA-cellulose columns. One major complication in the use of these columns is that some proteins which are positively charged at pH 6.8 bind to the DNA on the basis of charge. This problem was overcome by first adsorbing the serum to QAE-Sephadex to rid the serum of these proteins (primarily IgG). Human serum (50 ml) was passed through a QAE-Sephadex (A-50) column (4 x 20 cm) equilibrated in 0.01 M potassium phosphate, pH 6.5. The column was washed with the same buffer until all unadsorbed protein was removed, and then eluted with 0.01 M potassium phosphate, pH 6.5, containing 0.5 M NaCl. All fractions containing protein (QAE-Sephadex I) were pooled and dialyzed overnight against 0.01 M potassium phosphate, pH 6.8 containing 1 mM EDTA.

A DNA-cellulose column was equilibrated in the same buffer using DNA-cellulose containing 100 mg DNA per g of protein of QAE-Sephadex eluate. After application of the samples, the column was slowly washed with the same buffer until no more protein was removed. The bulk of the serum proteins (ca. 94%) were not retained by the column. The column was eluted with 0.01 M potassium phosphate, pH 6.8 plus 1 mM EDTA and 0.4 M NaCl. All fractions containing protein were pooled and dialyzed overnight at 4° against 0.01 M potassium phosphate, pH 6.8 plus 1 mM EDTA.

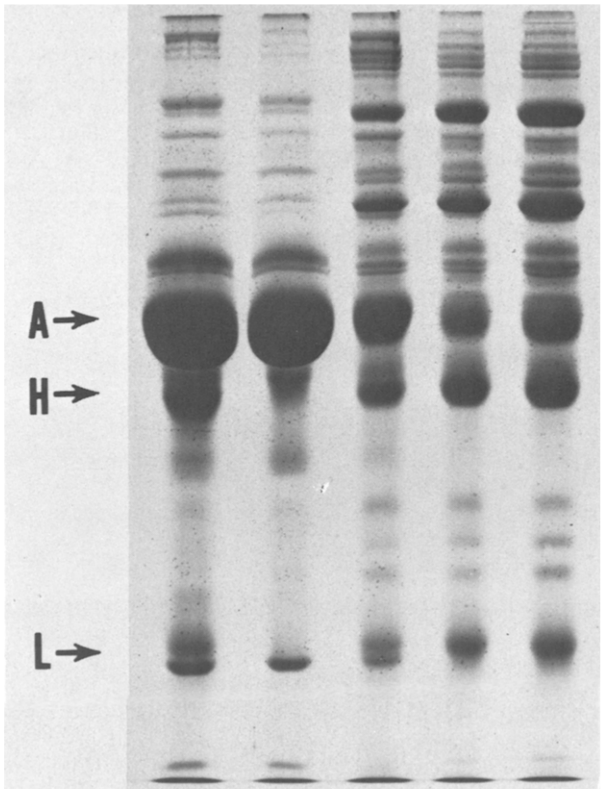

Fig. 1. SDS gel electrophoresis at each step of purification of DNA-binding proteins. The gel concentration was 10%. From left to right the samples are as follows: Whole serum (43 µg); QAE-Sephadex I (41 µg); DNA-cellulose I (41 µg); DNA-cellulose II (36 µg); QAE-Sephadex II (39 µg). The indicated bands are as follows: A, albumin; H, IgG heavy chain; L, IgG light chain.

This eluate (DNA-cellulose I) was passed through a DNA-cellulose column using a ratio of DNA-cellulose containing 100 mg DNA per 100 mg of protein applied. The same wash and elution buffers were used. All fractions containing protein were pooled and dialyzed overnight at 4° against 0.01 M potassium phosphate, pH 6.5.

This eluate (DNA-cellulose II) was again passed through a QAE-Sephadex column using the same conditions as in the first step. All fractions containing protein were pooled and dialyzed overnight at 4° against 0.01 M potassium phosphate, pH 6.8, plus 1 mM EDTA (QAE-Sephadex II).

Fig. 1 shows the analytical polyacrylamide slab gel electrophoresis

patterns in SDS of the proteins from each of these steps. The dominant protein in both the whole serum and the eluate from QAE-Sephadex I was albumin, and the latter fraction is deficient in IgG as evidenced by the strong decrease in the bands corresponding to the heavy and light chains of these molecules. The eluate from the first DNA-cellulose column shows a much different pattern with two high molecular weight bands predominating along with a decreased albumin component and a wide range of minor bands of heterogeneous molecular weight. IgG not removed by the QAE-Sephadex column appears in this and subsequent fractions. Rechromatography of this fraction on DNA-cellulose served to remove the bulk of the protein at the albumin position but the pattern of major and minor bands remained the same. Although a band is visible at the

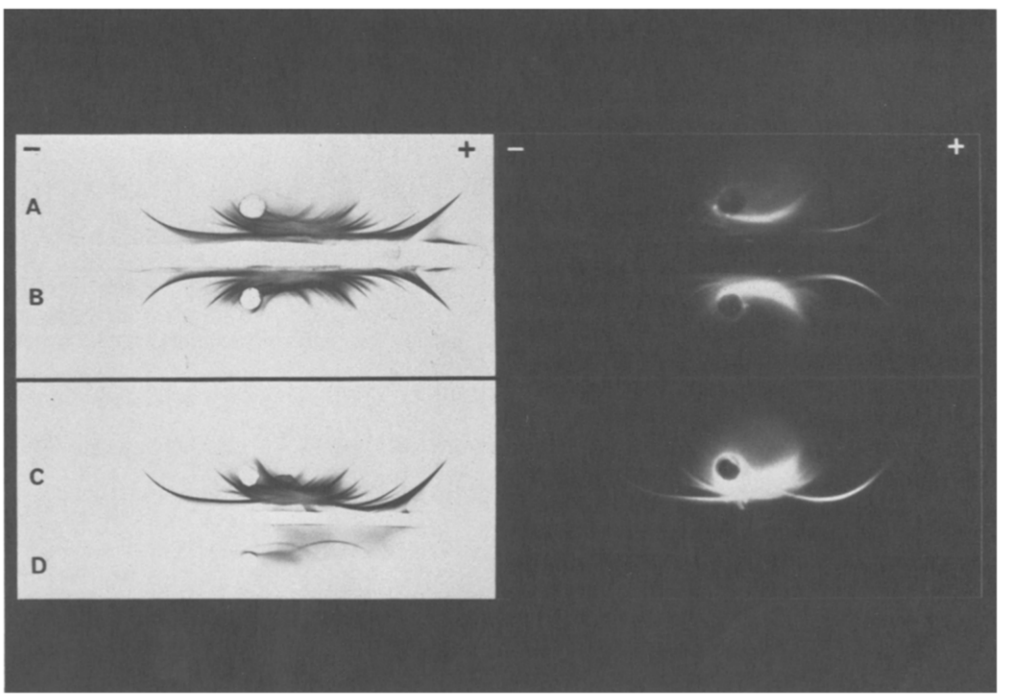

Fig. 2. Radioimmunoelectrophoresis of ^{125}I labeled QAE-Sephadex II (QSII) fraction. The slide on the left was stained with Amidoschwartz 10 B; the radioautogram resulting from exposure of the slide to Kodak Royal-X-Pan film is shown on the right. A. 1 µl ^{125}I-QSII at 0.1 mg/ml, 2.5 µl normal human serum. B. 2 µl ^{125}I-QSII, 2.5 µl normal human serum. C. 3 µl ^{125}I-QSII, 1.5 µl normal human serum. D. 4 µl unlabeled QSII at 1.2 mg/ml. The troughs both contained antisera to normal human serum (Behring Diagnostics).

albumin molecular weight position, Ouchterlony double diffusion analysis using anti-albumin antisera did not indicate the presence of albumin. The final QAE-Sephadex eluate also was little changed in protein pattern pointing out that these proteins are anionic and the DNA-cellulose is not simply isolating residual cationic proteins from the first QAE-Sephadex chromatography. The purification procedure to this point has been formulated with the one objective of isolating all the DNA-binding proteins in human serum preparatory to any subfractionation.

Immunological characterization of the DNA-binding proteins - The final fraction was examined by radioimmunoelectrophoresis after labeling with ^{125}I. Unlabeled human serum was added as a carrier. Examination of the stained slide and its radioautogram (Fig. 2) revealed that the major proteins of the final fraction migrate in the α_2 and β regions. This result is predicted by the chromatographic behavior of the proteins in the purification and verified that these proteins are not being isolated on the basis of overall net charge.

Protein from the second DNA-cellulose column was tested against thirty-nine different antisera on Ouchterlony double diffusion plates to determine if this fraction contained any known characterized serum proteins. The antisera tested were made against the following human proteins: immunoglobulins A,D,E,G, and M; transferrin, prealbumin; albumin; fibrinogen; β-lipoprotein; α-lipoprotein; ceruloplasmin; α_2-macroglobulin; complement components, $C1_s$, $C1_q$, C2, C3, C4, C5, C6, C8, C9; C1 inhibitor; C3-proactivator; α_1-antichymotrypsin; α_1-antitrypsin; inter-α-trypsin inhibitor; haptoglobin; β_2-glycoprotein I; β_2-glycoprotein III; α_2-HS-glycoprotein; α_1-T-glycoprotein; Zn-α_2-glycoprotein; α_1-acid glycoprotein; α_1B-glycoprotein; hemopexin; C-reactive protein; Gc globulins; and whole serum. The only precipitin reactions observed were with the antisera to IgG, α_1-antichymotrypsin, β-lipoprotein and whole serum. The β-lipoprotein was removed by the second QAE-Sephadex column. Examination of protein bands on calibrated SDS polyacrylamide gels had already indicated that neither the IgG nor the α_1-antichymotrypsin comprised a major fraction of the DNA-binding proteins.

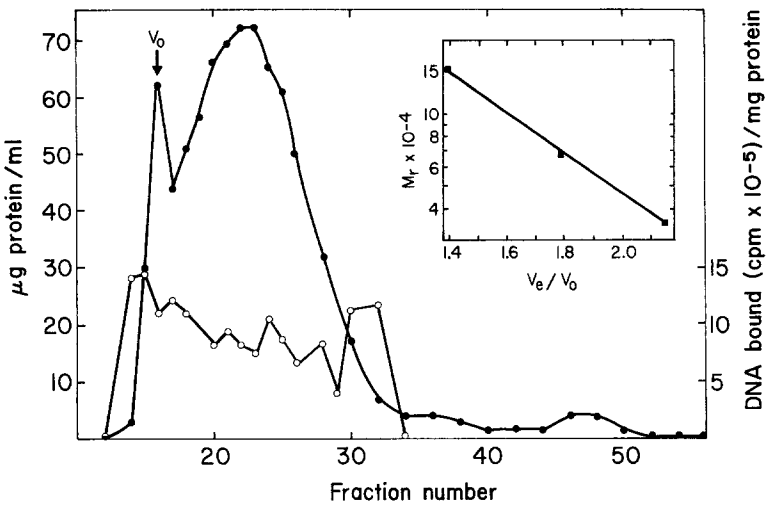

Fig. 3. Sephadex G-150 chromatography of DNA-cellulose II fraction. 2.1 mg of DNA-cellulose II fraction in 1 ml of 0.01 M potassium phosphate, pH 6.8, plus 1 mM EDTA, 10% glycerol, was loaded on top of a Sephadex G-150 column (1.6 x 46.5 cm) equilibrated in 0.1 M potassium phosphate, pH 6.5. 50 μl of each fraction (2 ml) were used to assay for DNA-binding activity. Inset, Whitaker (14) plot. The standards used were: human IgG (150,200); bovine serum albumin (68,000); pepsin (35,000).

Size and distribution of DNA-binding proteins - A sample from the second DNA-cellulose column was passed through a Sephadex G-150 column in an attempt to separate by size the different protein species seen on radioimmunoelectrophoresis (Fig. 3). There was greater than 75% recovery of DNA-binding activity after this column. The first peak of DNA-binding activity corresponded to the void volume protein peak indicating a molecular weight greater than 400,000. The rest of the activity was widely distributed over the remaining fractions suggesting that no major class of proteins isolated after the second DNA-cellulose column lacked specificity for DNA at least under the conditions of our "in vitro" assay.

The reduced proteins were accurately sized by analytical slab gel electrophoresis in SDS using 10% polyacrylamide concentration. At least two thirds of the proteins in the system were found to have molecular weights in excess of 70,000. The overall distribution of major and minor bands ranged from 22,500 (immunoglobulin light chains) to greater than 250,000. The major bands

have molecular weights of approximately 126,000 and 94,000.

DISCUSSION

This communication describes the isolation of DNA-binding proteins from human serum. The available evidence suggests that the DNA-binding proteins isolated according to our procedure may well comprise a unique series of molecules distinct from previously characterized serum proteins. Ouchterlony double diffusion analysis with thirty-nine antisera to known serum proteins gave precipitin lines only with anti-IgG and anti-α_1-antichymotrypsin, neither of which comprised a major fraction of the DNA-binding proteins. Analysis by radioimmunoelectrophoresis revealed that the dominant proteins of the DNA-binding fraction consist of acidic proteins migrating in the α_2-β regions.

The removal of IgG by QAE-Sephadex chromatography may bias the final DNA-binding protein fraction against basic proteins with true specificity for DNA. This problem cannot be overcome at present since the presence of large amounts of IgG decreases the capacity of the DNA-cellulose column for the acidic components. Another aspect, regarding the specificity of the proteins isolated here, is the fact that we are using calf thymus DNA to isolate human DNA-binding proteins. It is known that species specificity for DNA is resident in some nuclear acidic proteins (12). Serum components with absolute specificity for human DNA would not be detected by our methods.

Some of the classic methods used for the isolation and characterization of antibodies to DNA do not appear to be useful in the detection or assay of human serum DNA-binding proteins. Thoburn et al (2) reported the absence of any DNA-binding proteins in normal human serum based on the hemagglutination and precipitation techniques which would require divalent binding sites on the protein molecule as are found on antibodies. We were unsuccessful in our attempts to measure activity using the ammonium sulfate precipitation assay (13) that is routinely used to measure antibodies to DNA which are found in the sera of patients with systemic lupus erythematosus.

No attempt has been made to correlate the existence of DNA-binding pro-

teins in serum with biological function. Indeed, the apparent presence of several protein species differing in size and charge would suggest more than one possible function. There are of course a number of possible functions for these proteins. Some may exist simply as scavengers, with broad specificity, removing native or denatured DNA from the serum. This interaction may be more specific if some proteins serve as carriers for native DNA, perhaps even recognizing particular sequences within the DNA molecule. This last alternative touches on the possible involvement of these proteins in gene regulation. Their function may be analogous although not necessarily identical to those of DNA-binding proteins associated with the nuclear chromatin or found in the cytoplasm of mammalian cells. Such a regulatory role for protein existing in physiological fluids has already been postulated for the DNA-binding proteins in cerebrospinal fluid (1).

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