Brief Definitive Report

DNA-BINDING PROPERTY OF Sm NUCLEAR ANTIGEN*

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Antibodies to nuclear antigens are detected frequently in the sera of patients with systemic lupus erythematosus (SLE) and related autoimmune diseases. These antibodies have multiple immunochemical specificities and they react with different macromolecular components of cell nuclei (1), among which are nonhistone or nuclear acidic proteins. The first nuclear acidic protein characterized immunochemically with the aid of spontaneously occurring antibodies was called Sm nuclear antigen (2). Subsequently, other nuclear acidic protein antigens have been identified in a similar manner and include a nuclear ribonucleoprotein antigen reactive with sera of patients with SLE and mixed connective tissue disease (3) and other acidic nuclear proteins which are reactive with sera of patients with Sjögren's syndrome, scleroderma, and rheumatoid arthritis (4–6).

Many nuclear acidic proteins have been shown to have the property of binding to DNA and to play a role in the control of transcription of DNA to RNA in prokaryotic and eukaryotic systems (7). In the studies reported here, we have shown that tissue extracts containing Sm nuclear antigen have the property of binding to single-strand DNA but not to double-strand DNA.

Materials and Methods

Source of Sm Antigen   The nuclear origin of the Sm antigen had been established in previous studies (2, 4). The antigen was present in rabbit thymus acetone powder (8) and because this was readily available commercially (Pel-Freez Bio-Animals, Inc., Rogers, Ark.) it was used as the source of Sm antigen. 60 mg of this powder was extracted in each 1 ml of phosphate-buffered saline (0.15 M NaCl, 0.01 M PO_4, pH 7.3) by gently stirring the suspension at 4°C for 4 h After centrifugation at 10,000 rpm for 10 min, the supernatant solution contained protein varying from 10 to 15 mg/ml Partial purification of Sm antigen was obtained by salting out between 35 and 60% saturation with ammonium sulfate. This fraction contained all Sm antigenic activity but only one-half of the total protein and was used as the source material in further studies.

Immunological Detection of Sm Antigenic Activity   Sera from patients with SLE containing high titers of antibody to Sm antigen were used as reagents to detect presence of Sm antigen in isolated fractions of rabbit thymus extract. These sera had no detectable antibody activity to other known nuclear antigen-antibody systems, including DNA, deoxyribonucleoprotein, nuclear ribonucleoprotein, and nuclear antigens A and B reported in Sjögren's syndrome (4). In this sense, these SLE sera were "monospecific" for antibodies to Sm. The immunological assays used were immunodiffusion and inhibition of passive hemagglutination In the latter test, tanned sheep erythrocytes were coated with rabbit thymus extract according to a procedure that resulted in complexing of Sm antigen to the erythrocytes (9) Presence of Sm antigen in isolated fractions was determined by inhibition of hemagglutination.

Binding of Sm Antigen to DNA. Double-strand highly polymerized calf thymus DNA (Worthington Biochemical Corp , Freehold, N. J.) was dissolved in 50 mM potassium phosphate buffer, pH 8.0, and complexed to cellulose by ultraviolet light activation according to the method of Litman (9) 2 grams of DNA-cellulose were swollen in 10 mM phosphate, 1 mM EDTA (pH 6.8) buffer and 200 mg of rabbit thymus extract (35–60% ammonium sulfate preparation) was added to the suspension in a final 5-ml volume. The mixture was incubated at room temperature for 60 min, then poured into a column for elution chromatography. The column was washed with starting buffer until no protein was detected by absorbance at 280 nm. Then stepwise elution was performed with increasing concentrations of NaCl, 50 mM, 200 mM, 500 mM, 1 M, and 2 M in phosphate buffer, pH 6.8. Each pool of eluate was dialyzed against physiological pH buffers and tested for Sm antigenic activity after concentration. Total protein was 2.8 mg/ml in the first pooled fraction and varied from 0.09 to 0.420 mg/ml in the subsequent fractions

Single-strand DNA absorbant was made by complexing heat-denatured DNA with cyanogen-bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) according to the method of Poonian et al. (10) After the coupling procedure, the mixture was first washed extensively with 1.0 M ethanolamine, pH 8.0, and then with 50 mM phosphate buffer, pH 8.0 and pH 6.0, to remove unbound nucleic acid. Mixing of rabbit thymus extract to single-strand DNA-Sepharose was carried out in 50 mM phosphate buffer, pH 6.0, and elution was performed stepwise with 500 mM phosphate, pH 6.0, followed by sodium thiocyanate, 3.5 M at pH 6.8. Fractions were pooled and dialyzed back to physiological buffers for immunological assays.

Binding to Isotopically Labeled DNA   A radioimmunoassay method was adapted to determine if protein fractions from rabbit thymus extract would bind with DNA (11) For binding with double-strand DNA, native ^{3}H-labeled *Bacillus subtilis* DNA was used and for binding with single-strand DNA, sonicated, heat-denatured DNA was used. Extent of binding to each DNA preparation was determined by the amount of radioactivity retained on the filter compared to appropriate controls

Results

Binding of Sm Antigen to Single-Strand DNA. The result obtained with use of single-strand DNA-Sepharose as an adsorbant is illustrated in Fig. 1. With the equilibrating buffer of 0.05 M phosphate, pH 6.0, there was a large unadsorbed peak of material with 280 nm absorbancy. Elution with 0.5 M phosphate, pH 6.0, released another sizable peak and final elution with the chaotropic buffer 3.5 M sodium thiocyanate, pH 6.8, released the remaining material consisting of a smaller peak of 280 nm absorbing material. Fractions from the three distinct peaks were pooled and tested for Sm antigenic activity by immunodiffusion (Ouchterlony) against a standard reference serum containing antibody. Only the eluate one (E1) pool showed a precipitin line of identity with the precipitin line of the original rabbit thymus extract.

Further confirmation of the presence of Sm antigenicity in E1 pool was obtained by inhibition of passive hemagglutination (Fig. 2). In the control panel, hemagglutination of antigen-sensitized cells by a serum containing antibody to Sm antigen is present up to the fifth well. This is inhibited by addition of thymus extract at a concentration of 1 mg/ml. The unadsorbed fraction (UF) did not show inhibition but the E1 pool was inhibitory at 0.005 mg/ml. In a number of experiments of this kind, it was estimated that Sm antigen was purified about 10- to 20-fold.

In contrast to the ability of single-strand DNA to bind Sm antigen, double-strand DNA appeared not to have this capacity. This was the case whether the equilibrating buffer used for mixing thymus extract with double-strand DNA-cellulose was the same as that used with single-strand DNA adsorbant or was 0.01 M phosphate, pH 6.8.
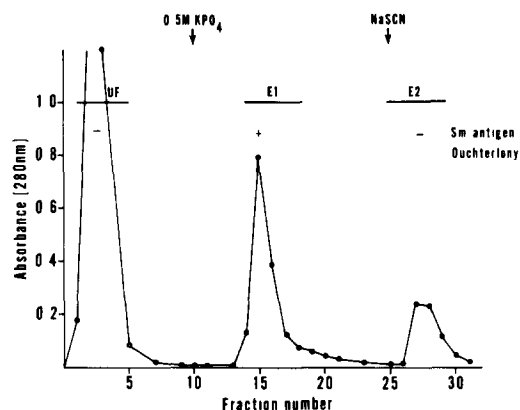
FIG. 1. Affinity chromatography of rabbit thymus extract on single-strand DNA-Sepharose adsorbant. The first peak represented unadsorbed fraction coming off with equilibrating buffer of 0 05 M phosphate, pH 6.0. Elution with 0.5 M phosphate, pH 6.0, and 3 5 M sodium thiocyanate, pH 6.8, resulted in two fractions, eluate one (E1) and two (E2). All fractions were pooled, neutralized, dialyzed, and tested for Sm antigen which was present only in E1.
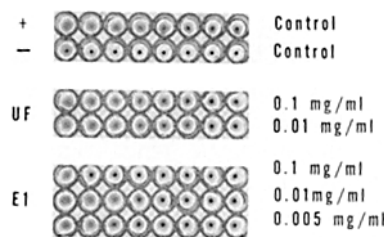

FIG. 2. Inhibition of passive hemagglutination Control panel shows hemagglutination of antigen-sensitized cells by a SLE serum containing anti-Sm antibodies. Addition of E1 inhibited hemagglutination, whereas UF did not

Binding to Isotopically Labeled DNA. Cellulose ester membrane filter radioassay provided another method for demonstrating binding to DNA of various fractions from the absorbant columns. A representative study is shown in Fig. 3. The E1 fraction previously demonstrated to contain Sm antigenic activity was able to bind in a dose-response fashion to single-strand DNA and showed insignificant binding to double-strand DNA. The unadsorbed fractions were nonreactive.

Discussion

There is a large and growing interest in the acidic nuclear proteins because evidence has accumulated in support of the idea that two groups of chromosomal proteins, the histones, and the acidic proteins, are part of the regulatory mechanism controlling differential gene expression (7, 12, 13). Many acidic nuclear proteins have the capacity to bind to DNA. This binding involves noncovalent forces, and the binding capacity is very heterogeneous, involving homologous, heterologous, and double- or single-strand DNA (14, 15).
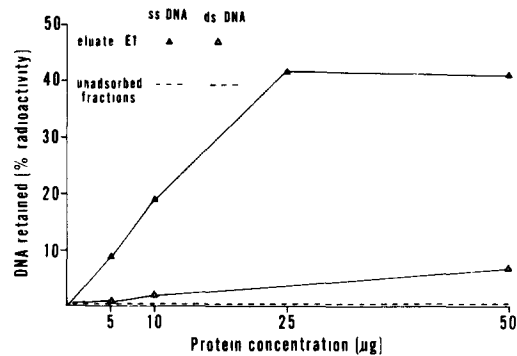
Fig 3   Membrane filter assay with isotopically labeled DNA Column fraction UF and E1 from single-strand (ss) DNA-Sepharose columns were reacted with ³H-labeled *B subtilis* DNA before filtering on cellulose membranes E1 bound to single-strand DNA in a dose-response fashion but not significantly to double-strand (ds) DNA. UF was unreactive

In this study, it has been demonstrated that an autoantibody in human disease is directed against a nuclear acidic protein which has the capacity to bind to DNA. There are two interesting features of the Sm nuclear protein antigen studied here. One is its preferential binding to single-strand DNA over double-strand DNA. Acidic nuclear proteins of this nature have been described in microorganisms (16) and in calf thymus (17). It has been postulated that this type of protein may act as DNA-unwinding proteins which decrease the stability of the double helix and allows for DNA replication and genetic recombination (7). In the first reported studies on the Sm antigen (2), preparations of calf thymus chromatin donated by Dr. J. H. Frenster, Stanford, Calif., were tested for Sm antigen. The antigen was detected in active chromatin but not in repressed chromatin.

Another interesting feature of Sm nuclear antigen is its presence in many tissues of the same organism and in different species (2, 3). The ubiquity of Sm antigen makes it unlikely as a protein regulating tissue differentiation. It is more likely to be analogous to other widely distributed acidic nuclear proteins which are suggested to regulate basic metabolic and transcriptional activities (18).

In previous studies (19), other investigators used an extract of calf thymus nuclei called extractable nuclear antigens and showed that this material bound to double-stranded DNA. It is difficult to determine if Sm antigen was included among the binding proteins since the reported studies did not include elution of material bound to DNA and analysis of the eluate.

The relationship between antibodies to nuclear acidic proteins and disease activity is unknown. Since some of the nuclear acidic proteins are DNA-binding proteins with a regulatory role in gene expression, the question obviously arises whether or not antibodies to these regulatory proteins might modify their in vivo activity. Some of these antibodies, like antibody to Sm, are highly restricted to one disease such as SLE, whereas antibodies to other nuclear acidic proteins are present in many diseases (1, 5). The reason or reasons for this discrimination in occurrence of autoantibodies is unknown. Finally, as demonstrated in this study, spontaneously occurring antibodies in human disease can

be used as specific reagents to isolate and study the properties of certain nuclear or cytoplasmic macromolecules which are present in trace amounts and which might be difficult to purify by purely physicochemical techniques.

Summary

Antibodies in the sera of patients with systemic lupus erythematosus reacted with a nuclear acidic protein called Sm antigen, and these antibodies were used as reagents to identify Sm antigen in preparative fractionation procedures. DNA affinity chromatography showed that Sm antigen was associated with nuclear protein fractions which had DNA-binding capacity. Evidence was also presented that Sm antigen showed preferential binding for single-strand DNA over double-strand DNA. These studies demonstrate that spontaneously occurring anti-nuclear antibodies in disease states may be used to study the properties of cellular proteins which are present in trace amounts.

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