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IDENTIFICATION OF A MAJOR HUMAN SERUM DNA-BINDING PROTEIN AS β1H OF THE ALTERNATIVE PATHWAY OF COMPLEMENT ACTIVATION

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Summary: One of the major proteins in human serum capable of binding DNA (designated DBP-1) has been identified as β1H of the alternative complement pathway. The two proteins exhibit the same molecular weight and amino acid composition. When compared directly using immunoassays, DBP-1 and β1H appear immunologically identical. Finally, although isolated as a complement component and not as a DNA-binding protein, β1H demonstrates an affinity for DNA comparable to that of DBP-1.

Introduction

DNA-binding protein-1 (DBP-1) is one of the major human serum proteins capable of binding to DNA (1). DBP-1 has been isolated to homogeneity and characterized with respect to physicochemical properties (2) as well as specific polynucleotide binding affinities (3). This report identifies DBP-1 as β1H, a regulatory protein of the alternative pathway of complement activation (4). β1H plays a critical role in the alternative complement pathway in that it regulates the formation and stability of the C3 and C5 convertase complexes, and thus controls the ability of the system to cleave C5 and form the membrane attack complex necessary to the cell-killing activity of the system (5).

MATERIALS AND METHODS

Purification of DBP-1: All operations were carried out at 4°C and all buffers contained 1mM 2-mercaptoethanol, 1 mM EDTA, and 0.1 mM phenylmethylsulfonylfluoride. Serum was obtained from the San Diego Blood Bank. The recently-described purification protocol for DBP-1 (3) was followed through the QAE-Sephadex

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chromatography, DNA-cellulose chromatography (step elution at pH 6.8) and the ammonium sulfate fractionation. DBP-1 was further purified as follows.

DNA cellulose chromatography (gradient elution at pH 7.8): The 0 to 40% ammonium sulfate fraction was dialyzed into 10 mM Tris-phosphate (pH 7.8) containing 0.05 M NaCl, 20% glycerol (v/v) and 1 mM NaN_3. The dialysate was applied to a DNA-cellulose column containing 2.5 mg of DNA per mg of protein and equilibrated in the dialysis buffer. After adsorption of the protein sample, the column was extensively washed using 5 ml of buffer per gram of DNA-cellulose. The adsorbed protein was eluted with a linear 0.05 to 0.4 M NaCl gradient (total volume = 15 ml of buffer per gram of DNA-cellulose). The fractions containing DBP-1 were determined by SDS-polyacrylamide gel electrophoresis. The peak tubes were pooled and concentrated by the addition of 1.5 volumes of saturated ammonium sulfate to 60% saturation. The precipitate was resuspended in 3 ml of 0.1 M Tris-HCl (pH 7.5) containing 0.2 M NaCl, 15% glycerol (v/v) and 1 mM NaN_3.

Sepharose 6B chromatography: The sample was applied to a Sepharose 6B column (1.6 x 80 cm) equilibrated in the same buffer. The DBP-1 was again monitored by SDS gel electrophoresis. The purified DBP-1 was found to be greater than 95% homogeneous as judged by SDS-polyacrylamide slab gel electrophoresis. A number of protease inhibitors are routinely employed throughout the purification procedure, to minimize the generation of DBP-1 split products. In particular the use of buffers containing the high osmolality additive, glycerol, in the later purification steps minimizes the appearance of a species of approximately 118,000 molecular weight.

Purification of β1H: β1H was isolated as previously described (4).

Electrophoresis: SDS-polyacrylamide slab gel electrophoresis was carried out using the system of Laemmli (6). The gels were stained using the procedure of Fairbanks et al (7). The following proteins were used as standards to calibrate the gels: Unreduced human immunoglobin G (IgG, 150,000); phosphorylase α (92,500); conalbumin (77,000); human IgG heavy chain (50,000); ovalbumin (43,000).

Immunoelectrophoresis was carried out on ethanol-washed microscope slides layered with 5 ml of 1% agar in 75 mM barbital buffer (pH 8.6). Electrophoresis was performed in the same buffer at 175 volts for 90 minutes. To prepare antisera to DBP-1, 480 μg of purified DBP-1 were injected subcutaneously into a New Zealand white rabbit in complete Freund's adjuvant. The injections were repeated at three-week intervals using incomplete Freund's adjuvant. The rabbit was then boosted at three-week intervals using 48 μg of purified DPB-1 in incomplete Freund's adjuvant. One week following the second injection and at three-week intervals thereafter sera were collected from the rabbit.

Nitrocellulose filter assay: The assay to determine DNA binding has been previously described (3). The reaction mixture contained 10 mM potassium phosphate buffer (pH 6.0) containing 1 mM 2-mercaptoethanol, varying amounts of [^{3}H] labelled Wil_2 DNA (194,000 cpm/μg) and the protein (1 μg) to be tested.

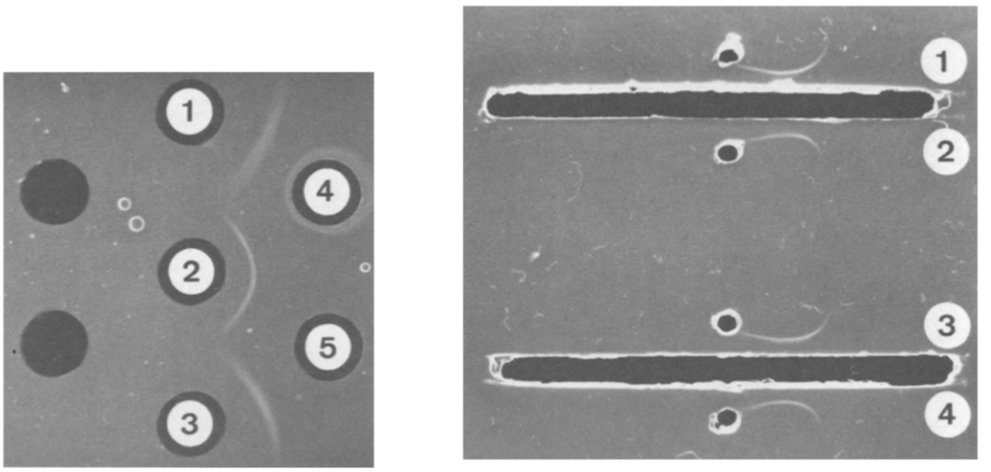

Figure 1. Left. Agar double-diffusion studies. Wells 1 and 3 contained human βlH and Well 2, DBP-1, all at a concentration of 0.47 mg/ml. Wells 4 and 5 contained antisera directed against DBP-1 and βlH respectively.
Right. Immunoelectrophoresis. Wells 1 and 3 contained human βlH and Wells 2 and 4, DBP-1. The upper trough contained antisera directed against βlH, and the lower trough, antisera directed against DBP-1.

RESULTS

Immunoassay of DBP-1 and βlH-globulin: Purified preparations of DBP-1 were found to react with antisera directed against βlH. A double diffusion study using human DBP-1 and βlH allowed a direct comparison of the two proteins as seen in Figure 1. There was a clear line of identity between the two proteins using antisera directed against either DBP-1 or βlH. When the two proteins were subjected to immunoelectrophoresis, both exhibited a single precipitin arc of β mobility, again using antisera directed against either DBP-1 or βlH (Figure 1).

Physicochemical properties: Both DBP-1 and βlH contain carbohydrate as determined by periodic acid-Schiff (PAS) stain of SDS gels. Both proteins have been reported to consist of a single polypeptide chain, and the reported molecular weights as determined by SDS-polyacrylamide gel electrophoresis were somewhat

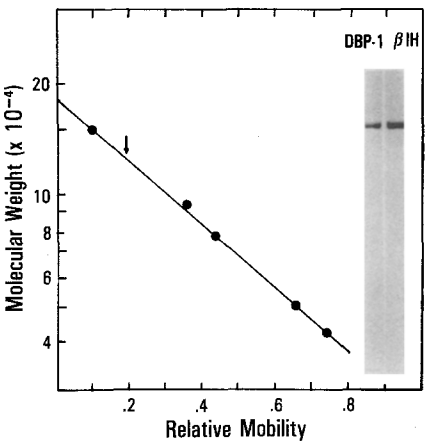

Figure 2. SDS-polyacrylamide slab gel electrophoresis of DBP-1 and βlH. Each sample contained 4 μg of protein. The gel concentration was 7.5%. The standards are as follows: unreduced immunoglobulin G, phosphorylase α, conalbumin, immunoglobulin G heavy chain, ovalbumin.

similar (within 15%) (2, 8). However glycoproteins may exhibit different apparent molecular weights depending on the gel concentration under such electrophoresis conditions. Thus these weights were determined for the two proteins under identical experimental conditions. As seen in Figure 2, the two proteins exhibit the same mobility. The results of four such experiments indicated a molecular weight for the two proteins of 130,500 ± 2700 at acrylamide concentrations of 7.5%. When a non-denaturing, alkaline polyacrylamide disc gel system is used, both proteins only partially enter 4 to 5% gels which may result from self-aggregation in the absence of 2-mercaptoethanol. Another major similarity between DBP-1 and βlH is their amino acid compositions (Table 1). Again a direct comparison was made by subjecting samples of DBP-1 and βlH to hydrolysis and analysis under identical conditions. Both proteins are relatively rich in proline and cysteine and contain little methionine.

DNA-binding properties: DBP-1 was isolated by a combination of chromatographic techniques with particular reliance on DNA af-

Table I. Amino Acid Composition of DBP-1[a]

Amino Acid	DBP-1 mol/100	βlH mol/100
Lysine	7.27	7.06
Histidine	2.52	2.37
Arginine	5.34	4.90
Aspartic acid	10.13	10.22
Threonine	6.97	6.60
Serine	7.12	6.95
Glutamic acid	12.43	12.49
Proline	9.81	9.96
Glycine	9.08	9.59
Alanine	3.46	3.60
Half cystine[b]	5.73	5.42
Valine	5.23	5.34
Methionine	1.35	1.40
Isoleucine	5.53	5.51
Leucine	5.33	5.36
Tyrosine	6.01	5.99
Phenylalanine	2.42	2.66

[a] Both samples were hydrolyzed for 24 hr prior to analysis.

[b] Half cystine was determined as cysteic acid after performic acid oxidation: DBP-1 (2); βlH (M.K. Pangburn, unpublished).

finity chromatography. It was of primary interest to determine if the βlH isolated without this selection for DNA-binding ability, did in fact bind to DNA. A nitrocellulose filter assay system was used to determine the equilibrium constants for the binding of double-stranded human lymphocyte DNA to both proteins. As seen in Figure 3, the equilibrium constant was calculated to be 2.1×10^{-5} M for each protein under the conditions described in

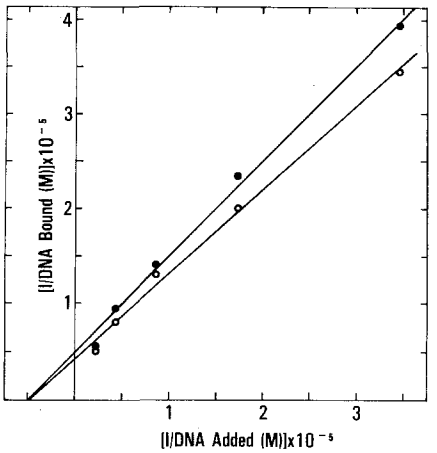

Figure 3. Determination of the equilibrium constants for the binding of [^{3}H] labelled double stranded Wil_2 DNA by DBP-1 (-●-) and βlH (-o-).

the Materials and Methods. The DBP-1 protein was slightly more active than the βlH but the difference was certainly within the range of variability seen for different DBP-1 preparations.

DISCUSSION

DBP-1, first characterized as a major human serum DNA-binding protein, has been identified as βlH of the alternative pathway of complement activation based on the following criteria. The two proteins exhibit apparent immunological identity when reacted with monospecific antisera under conditions of double diffusion or immunoelectrophoresis. Both are glycoproteins with similar amino acid compositions and with identical molecular weights as determined under denaturing gel electrophoresis. Finally, both are DNA-binding proteins. With the identification of DBP-1 as βlH, a new method is available for the isolation of this complement protein using DNA affinity chromatography and conditions that minimize the generation of split products. The method is feasible for large-scale isolation protocols, and in

fact, allows for the parallel isolation of complement factor B which also binds to DNA-cellulose (10) unlike the usual separate protocols for the isolation of these two complement proteins. The fact that DBP-1 and β1H exhibited a similar binding profile with the human lymphocyte DNA would indicate that DBP-1 does not represent a unique subset of β1H molecules that has DNA-binding activity. Thus two critical elements of the alternative complement pathway, β1H and factor B, have now been associated with the capability of binding polynucleotides with a specificity that is dependent at least in part on the conformation of the substrate and on its base sequence. It remains to establish that this ability relates to an in vivo cellular function for each of these proteins.

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