MICROHETEROGENEITY OF GLYCOPROTEINS

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Affinity chromatography and electroimmunoprecipitation can be combined to obtain analytical procedures with the following aims: To identify ligand-binding proteins, to characterize the binding with respect to structure and number of binding sites and the association constant, to quantify individual carbohydrate forms of microheterogeneous glycoproteins, and to predict the results of preparative affinity separations.

The following methods were developed to study glycoproteins with lectins, and they differ in the order in which proteins interact with lectins. In Method A the sample is incubated with lectin before analysis by crossed immunoelectrophoresis. In Method B free lectin or immobilized lectin is incorporated in an intermediate gel in crossed immunoelectrophoresis. In Method C free lectin or immobilized lectin is incorporated in the first dimension gel of crossed immunoelectrophoresis. By comparing with control experiments it is possible to identify and characterize protein-lectin interactions by differences in electrophoretic and antigenic behavior of the protein under study. Protein lectin interaction can then be studied by comparison of the electrophoretic and antigenic behavior of the proteins under study with the results of control experiments.

Using these methods we have found that the microheterogeneity of serum glycoproteins is remarkably constant in normal individuals, but is changed characteristically in disease.

Examples of quantitation of microheterogeneity forms of disease markers will be mentioned (eg. AFP, ferritin, AGP and other acute phase proteins) and their biological significance will be discussed.

Memo:

ELECTROPHORETIC PROPERTIES OF RCA-2 BINDING GLYCOPROTEINS IN HUMAN SERA AND ASCITES WITH HEPATOCELLULAR CARCINOMA

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Glycoproteins associated with hepatocellular carcinoma,which have Gal or GalNAc at their non-reducing end, were determined by hemagglutination inhibition test, in patints' sera with digestive diseases, and these glycoproteins were purified with RCA-2-agarose and anti-whole human serum-Sepharose 4B affinity chromatographies. Their electrophoretic properties were analysed with SDS-polyacryl amide gel electrophoresis(SDS-PAGE).

For the determination of RCA-2 lectin binding glycoproteins, 25µl of sample which had been appropiately diluted with Gelatin Veronal Buffer(GVB) were mixed with 25µl of human O-type RBC (1.5% Ht) and 25µl of RCA-2 lectin(50µg/ml), and the mixture was incubated at 37°C for 60 min to check hemagglutination inhibition. 10 ml of ascitic fluid(170 mg protein) which had been obtained from a patient with hepatocellular carcinoma were applied to a column(1X14 cm) of RCA-2-agarose(4 mg/ml of RCA-2) and RCA -2 binding glycoproteins were eluted with 0.3M β-lactose after washing the column with GVB. The eluate was then passed through a column(1.6X28 cm) of anti-whole human serum-Sepharose 4B(15 mg /ml of IgG) to absorb normal serum components. Glycoproteins thus prepared were subjected to SDS-PAGE(7.5%) electrophoresis.

RCA-2 binding glycoproteins in the serum were detected in 10/21(47.6%) of hepatocellular carcinoma, 9/22(40.9%) of liver cirrhosis, 1/20(5.0%) of chronic hepatitis, 1/10(10%) of acute hepatitis and 18/23(78.3%) of malignant digestive diseases other than hepatocellular carcinoma. 160 ml(2.7 g of protein) of ascites were applied to the affinity chromatographies successively and 3.6 mg of glycoproteins were prepared.

SDS-PAGE of the preparation showed only one main band(48K daltons).

Memo:

SEPARATION AND IDENTIFICATION OF DIFFERENT MOLECULAR SPECIES OF HUMAN α-FETOPROTEIN BY DOUBLE AFFINITY ELECTROPHORESIS WITH CONCANAVALIN A AND LENS CULINARIS HEMAGGLUTININ A

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Human α-fetoprotein(AFP) in body fluids is usually composed of various proportions of distinct molecular species with different affinities to concanavalin A(con-A) and Lens culinaris hemagglutinin A(LcH-A). This was demonstrated by isolation of each molecular species with Con A-Sepharose and LcH Gel in column chromatography and by crossed-immuno-affino-electrophoresis for determination of dissociation constants of AFP-lectin complexes.

AFP_1 had no affinity to either con-A or LcH-A, AFP_2 strong affinity to con-A but weak or no affinity to LcH-A, and AFP_3 strong affinity to both of the lectins. AFP_2 was a major component of cord serum AFP, and AFP_2 and AFP_3 were variable components of AFP from hepatocellular carcinoma or its cultured cell line(HuH-7). An additional species of AFP found in the medium of HuH-6 C1-5 had weak affinities to both of con-A and LcH-A. These molecular species of AFP were also separated and identified from their relative mobilities by double affinity electrophoresis with con-A and LcH-A, in which these lectins in separate agarose gels were connected serially in alternate orders(LcH-A → con-A and con-A → LcH-A). Simplicity of this method for one-step separation of major molecular species of AFP in biological materials was thus demonstrated. This work was supported by Grant-in-Aid from the Ministry of Education, Science and Culture(57570836).

Memo:

CROSSED IMMUNOELECTROPHORESIS FOR THE DETECTION OF SPLIT PRODUCTS OF THE THIRD COMPLEMENT IN DENGUE HEMORRHAGIC FEVER. I. OBSERVATION IN PATIENTS' PLASMA

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Crossed Immunoelectrophoresis (CIEP) was applied to detect the products of the third component of complement (C_3) activation in plasma of patients who suffered from Dengue Hemorrhagic Fever (DHF) which is caused by dengue viruses types 1,2,3 and 4.

First dimension electrophoresis was performed using 5x10 cm glass slides with 6 ml of 1% agarose in 0.01 M EDTA-Tris buffer pH 8.6. Samples, 1 µl for each test, were placed in to 1 mm punched wells. The electrophoresis was done by using 10 v/cm in 4°C for 60 min. Second dimension electrophoresis was run perpendicularly in rabbit anti-C_3 serum containing agarose and electrophoresis was performed by using 2v/cm 4°C overnight. Normal human serum treated with inulin, a known C_3 splitting agent, was used as positive control in all tests.

C_3 split products (C3sp) were demonstrated in severely ill patients clinically classified as the grade III and grade IV, and rapidly disappeared during convalescent phase. The appearance of C3sp correlated well with the signs of shock, and this electropherogram of C3sp could be used as a parameter reflecting immunological activity in this disease.

Memo:

AFFINOPHORESIS OF TRYPSINS. ELECTROPHORESIS OF TRYPSINS IN THE PRESENCE OF A SOLUBLE POLYELECTROLYTE BEARING AFFINITY LIGAND.

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We devised a new separation technique for the protein, "affinophoresis", which is based on its specific affinity and utilizes electrophoresis. This technique requires a carrier molecule, "affinophore", which contains both an affinity ligand for a certain protein and many charges, either positive or negative, in order to migrate rapidly in an electric field. When a mixture of proteins is electrophoresed in the presence of the affinophore, the protein having an affinity with the ligand will form a complex with the affinophore. This results in a change in the apparent electrophoretic mobility. If the protein is sufficiently accelerated, we can separate it from other materials.

A cationic affinophore for trypsin was prepared. Soluble dextran (MW 10,000) was coupled with a DEAE-group and m-aminobenzamidine, a competitive inhibitor of trypsins. Electrophoresis of trypsins from several origins on agarose gel plates in the presence of the affinophore showed that affinophoresis actually occurred. The electrophoretic mobilities of trypsins increased towards the cathode, the same direction as the affinophore movement. The presence of leupeptin and treatment of the trypsins with TLCK suppressed the effect of the affinophore. Streptomyces griseus trypsin, contained in Pronase, was easily separated and detected.

This procedure is distinct from affinity chromatography and so-called affinity electrophresis in that the support of the affinity ligand moves, and has advantages especially for analytical purposes: for example, the detection of specific molecules regardless of their isoelectric points.

Memo:

This abstract is the same as PS-3-27.

HETEROGENEITY OF RABBIT ANTI-DNP ANTIBODY STUDIED BY TWO-DIMENSIONAL AFFINITY ELECTROPHORESIS

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Rabbit anti-Dnp antibody was separated two-dimensionally in the first direction by isoelectric focusing(IEF) and in the second direction by affinity electrophoresis(AEP).

Rabbits were immunized with Dnp-human-γg-globulin. The anti Dnp antibodies were purified by ammonium sulfate fractionation followed by affinity chromatography with a Dnp-lysyl-Sepharose column. The obtained antibody preparations were composed of almost only IgG species in tests by SDS-disc electrophoresis, Ouchterlony procedure, and immunoelectrophoresis. IEF was carried out in 5% polyacrylamide gel containing 6 M urea, 10% sucrose and carrier ampholyte pH 4.0 - 9.0. AEP was carried out with the buffer system of Reisfeld et al. in the presence of Dnp- or Tnp-polyacrylamide conjugate.

Anti-Dnp-antibodies were separated into over 50 distinct IgG-spots. All the spots appeared to be homogeneous. They were divided into 8 - 10 groups according to the extent of their affinity to Dnp- and Tnp-haptens. While the affinity of every group to Dnp-hapten was different from those to the heterologous Tnp-hapten, the affinity of every spots belonging to each group changed at the identical rate. This indicated each group may be composed of a monoclonal antibody. These antibody groups have 2 to 7 times stronger affinity to Dnp-hapten than to Tnp-hapten except one group, which has 1.6 times stronger affinity to Tnp-hapten. Dissociation constants calculated by the original affinity equation amounted to 2×10^{-6}M to 1×10^{-4}M for Dnp-hapten and 1.4×10^{-5} to 1×10^{-4}M for Tnp-hapten.

Memo:

This abstract is the same as PS-3-16.