BINDING SPECIFICITY OF TRANSFERRIN RECEPTORS ON CULTURED TUMOR CELLS

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It has been shown recently that transferrin receptors are found in abundance on many tumor cells and may be a useful surface marker for cell proliferation. The purpose of this paper is to examine the binding specificity of transferrin receptors on cultured tumor cells with respect to human transferrin with different iron content.

(Materials and method) Human established cell lines (K562, H160, U937, Chang liver cell) were maintained in RPMI 1640 with 10% fetal calf serum. Transferrin was purified from pooled human plasma and the iron saturation of transferrin was assesed by 6M urea/polyacrylamide slab gel electrophoresis. Transferrin receptor was assayed by Scatchard plot binding study using ^{125}I labled transferrin.

(Results) 1. The number of transferrin receptors on K562, HL60, U937, and Chang liver cell, was 5×10^5, 3.9×10^5, 0.6×10^5, 1.0×10^5 per cell, respectively. 2. Association constant (Ka) of binding of transferrin receptor using diferiic transferrin was around 2.0×10^8 L/M. 3. Apotransferrin and two forms of monoferric transferrin (N-site bound and C-site bound), however, showed less than 10% of binding of diferric transferrin. 4. Neuraminidase treatment of diferric transferrin did not alter the binding affinity against transferrin receptors.

These results suggests that the transferrin receptor on cultured tumor cells only recognizes diferric transferrin.

Memo:

EXISTENCE OF MYOGLOBIN IN CHICKEN GIZZARD MUSCLE AND COMPARISON OF Mb BETWEEN DYSTROPHIC AND NORMAL CHICKENS

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The purpose of this paper was to confirm the existence of myoglobin(Mb) in chicken gizzard muscle, which was regarded as a smooth muscle, and to study the difference of Mb between normal and dystrophic chickens.

Mb was isolated from skeletal muscles by Singer's method, and Sephadex G-75 and DEAE column chromatography. Identification of Mb was done by spectrophotometry, polyacrylamide gel electrophoresis. A heme-protein was isolated by the same method from gizzard muscles of normal chicken, and was compared with Mb of skeletal muscle in physico-chemical properties and antigenicity. The antisera was raised in rabbits immunized with Mb of chicken skeletal muscle.

The Mb isolated from normal chicken skeletal muscles showed Mb-specific plateau effect at 580-610 nm by spectrophotometry (pH 5.4). By electrophoresis, 4 subfractions were obtained, whose mobilities were obviously distinct from hemoglobin. The heme-protein, probable Mb, isolated from normal chicken gizzard muscle was identical with Mb of normal chicken skeletal muscle in its absorption spectrum and electrophoretic mobility. Immunochemically, the heme-protein of gizzard muscle showed a single precipitin line with the antisera, which fused with the line between Mb of normal chicken skeletal muscle and the antisera.

Furthermore, Mb was isolated from skeletal and gizzard muscles of dystrophic chickens in the same way, and was found identical with Mb of normal chickens.

Memo:

CHARACTERIZATION OF MONOMER AND DIMER FERRITIN

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Ferritin is iron-storage protein consisting of an outer protein shell of mol. wt. 450000 with a central cavity containg a variable amount of iron, deposited as a microcrystalline ferric hydroxidephosphate complex. The protein component is composed of 24 subunits each of mol. wt. 19000 and 21000. When isoelectric-focusing techniques are used, isoferritin between tissue ferritins obserbed.

The horse spleen ferritin from different commercial sourses all showed at last three bands of different mobilities when examined by polyacrylamide gel electrophoresis. These bands are monomer, dimer and oligomer ferritin. Monomer and dimer ferritins purified from commercial pure ferritin fraction by preparative polyacrylamide gel electrophoresis and chromatography on Sepharose 6B. Incorporation of ^{59}Fe into dimer ferritin was consistently lower than that of the monomer ferritin (1/4 -1/5). The ferritin of higher iron content generally increased ^{59}Fe-incorporation. However dimer were independent of iron content. The most effective reagent to cleave the dimer to monomer were mercaptoethanol and dithiothreitol that cliave disulfide bonds. 0.5% mercaptoethanol treat had effect on the dimer of 50%, but residual dimer had no effect to cleave to monomer in the second time treat. The ratio of dimer to monomer of isoferritin (pI 3.97, 4.10, 4.19, 4.28, 4.31) estimated by polyacrylamide gel electrophoresis. The pI 3.97, 4.31 fractions were not contained to dimer, and other pI fractions were different dimer content.

Memo:

ELECTROPHORETIC ANALYSIS OF LIVER SPECIFIC LIPOPROTEIN(LSP)

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Immune reactions to hepatocytes membrane play an important role in liver cell injuries on chronic active hepatitis. Liver specific lipoprotein(LSP, Meyer,et al.) is known as one of the membrane lipoprotein which is observed on hepatocyte membrane only. But the biochemical nature of LSP is still unkown. Electrophretic analysis of LSP was performed.
Method: LSP was prepared by the gel filtration with Sephadex-G-100 and Sepharose 6B from supernatant of rat liver homogenate centrifuged at 105,000g. For electrophoretic analysis LSP was dialyzed with Tris/HCl containing Triton X-100 or deoxycholate(DOC). Polyacrylamide gradient(4-30%) gel electrophoresis(PAGE), SDS-PAGE and isoelectoric focusing(IEF) were done.
Results: Antigenicity of LSP did not alter after dialysis on immunodiffusion test to anti-LSP rabbit serum. DOC-treated LSP was electrophoresed successfully on PAGE and thirteen bands in molecular weight range between 700,000 and 50,000 was observed. On SDS-PAGE LSP was separated at least 14 bands in molecular weight range between 170,000 and 18,000. By IEF DOC-treated LSP was electrofocused in the range from PI. 7.35 to 9.59. On the contrary LSP was not electrophoresed on PAGE and IEF without DOC-treatment.
Discussion: By dialysis with Tris/HCl containing 1% DOC LSP was well analyzed electrophoretically without loss of its antigenicity to antibody. Using these methods there is an possibility of further purification and clarification on the nature of LSP.

Memo:

OPTIMIZING ELECTROPHORETIC METHODS FOR ANALYSIS OF GENETIC ORIGINS IN GRAIN AND FOOD SAMPLES

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Electrophoretic methods have a lot to offer the agricultural and food industries in the ability of these procedures to determine the genetic identity of agricultural products such as seeds or the origin of ingredients in foods. This approach takes advantage of the distinctive composition of key groups of proteins. Synthesized as direct gene products ("semantic molecules"), the proteins retain the distinctive chemical imprint of the species or variety of the organism in which they were formed. The important task is to determine what combination of procedures (extraction and electrophoretic methods) will most readily provide the information required.

Many questions may be answered by these methods. For example, has vegetable protein been used in this sample of cheese or processed meat? What type of fish is this fillet? What variety of wheat is in this flour? Is this barley seed true to label? Are these off-type oat plants wild oats or out-crosses?

All these questions can and have been answered. In many cases, a procedure devised to provide optimum results has proved inadequate when a new combination of genotypes has been presented. Established methods thus need to have variations that can be invoked for these difficult cases. Examples and recommendations provided to illustrate a range of applications will include the identification of closely related varieties of wheat, characterization of off-type plants in oat and wheat crops, quantitation of wheat and rye in bread mixes before and after baking, and fish-species identification.

Memo: