Electrophoretic Analysis of Microsomal Smooth Membrane Proteins in Rat Liver and in Morris Hepatoma 5123C

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SUMMARY

Smooth microsomal cell membranes were isolated by density gradient centrifugation from normal rat liver and from the well-differentiated Morris hepatoma 5123C. The proteins were then extracted from the smooth membrane fraction and compared on acrylamide gel by the split-gel technique and by coelectrophoresis of dually labeled proteins. This analysis revealed the lack of two liver membrane components in the membrane of the hepatoma. The change of protein composition of the cell membrane of a well-differentiated liver tumor is discussed briefly.

INTRODUCTION

A number of findings point to cell membranes as structures undergoing fundamental changes during neoplastic transformation. Lack of contact inhibition, random growth, appearance of new antigens, increase in membrane rigidity, changes in membrane charge density, and changes in chemical composition with respect to membrane glycolipids and glycoproteins all point to such a cellular alteration as a key event in carcinogenesis (9).

Unfortunately, at present we are not familiar enough with the structure of cell membrane to establish and to evaluate its changes during neoplastic transformation from a molecular point of view. Although cell membranes isolated from normal and neoplastic tissues have been studied and compared extensively, much of our present knowledge on this matter has been derived from microscopic studies and from biochemical studies on lipid and sugars.

A comparison of the mitochondrial membrane proteins in rat liver and hepatomas showing a major protein band present in the liver, which is missing in the tumor, has been carried out recently by Chang et al. (2) with acrylamide electrophoresis. The present report describes a comparative electrophoretic analysis of microsomal membrane proteins isolated from normal liver of Buffalo rats and from the well-differentiated, slowly growing Morris hepatoma 5123C. Thus small but clear-cut differences of protein composition have been demonstrated.

MATERIALS AND METHODS

Microsomal membranes were obtained by sedimentation of the microsomal fraction at the buoyant density on sucrose gradients according to the method of Dallner et al. (4). The membrane fraction floating on 1.3 M sucrose was used for electrophoretic analyses. In preliminary experiments the fraction to be used was checked by electron microscopy. It revealed characteristic small, smooth vesicles (Figs. 1 and 2). NADH-cytochrome c reductase values were measured on total homogenate and on the endoplasmic reticulum membrane fraction. Measures have been carried out according to the method of Sottocasa et al. (8). Enzyme level (μmoles of NADH oxidized per min per mg of protein) increased about 5-fold in the smooth membrane fraction as compared with the total homogenate for either the liver or the tumor.

Lipid-free membrane proteins were obtained as follows. The membranes were solubilized by addition of 4 volumes of a 1:1 mixture (v/v) of 3% non-ionic detergent (Triton N-101) in water and glacial acetic acid. The proteins were then precipitated with 5% trichloroacetic acid and collected by centrifugation. The sediment was washed with ethanol and ethanol/ether (1:1) at 65° and then solubilized with 8 M urea according to the method of Reisfeld et al. (7). This procedure has good resolving power for membrane proteins as compared with anionic systems with and without sodium dodecyl sulfate. Split-gel electrophoreses were carried out by substituting the sample gel with 2 semisections of prepolymerized gels loaded with the 2 proteins to be compared. Labeling of isolated membrane proteins with dimethyl sulfate (New England Nuclear, Frankfurt/Main, West Germany) was carried out according to the method of Kiehn and Holland (6), replacing sodium dodecyl sulfate of the original procedure with the non-ionic detergent Triton N-101 to avoid alteration of the intrinsic charge density of the labeled proteins. Morris hepatoma membrane proteins were labeled with dimethyl sulfate-14C, liver membrane proteins with dimethyl sulfate-3H. Double-labeled proteins (specific activity, 500 to 1000 cpm/μg) were subjected to acrylamide electrophoresis at pH 4.5 in 8 M urea according to the method of Reisfeld et al. (7). This procedure has good resolving power for membrane proteins as compared with anionic systems with and without sodium dodecyl sulfate. Split-gel electrophoreses were carried out by substituting the sample gel with 2 semisections of prepolymerized gels loaded with the 2 proteins to be compared. Labeling of isolated membrane proteins with dimethyl sulfate (New England Nuclear, Frankfurt/Main, West Germany) was carried out according to the method of Kiehn and Holland (6), replacing sodium dodecyl sulfate of the original procedure with the non-ionic detergent Triton N-101 to avoid alteration of the intrinsic charge density of the labeled proteins. Morris hepatoma membrane proteins were labeled with dimethyl sulfate-14C, liver membrane proteins with dimethyl sulfate-3H. Double-labeled proteins (specific activity, 500 to 1000 cpm/μg) were subjected to acrylamide electrophoresis at pH 4.5 in 8 M urea-containing gels, and the gels were sectioned with a Savant Autogeldivider and counted according to the method of Maizel (5). The recovery of input radioactivity was approximately 45%. All the chemicals were reagent grade and were supplied by Carlo Erba Co., Milan, Italy. Special chemicals were obtained from Sigma Chemical Co., St. Louis, Mo.

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RESULTS

Duplicate acrylamide gels, stained with Amido black, loaded with the split-gel technique, with Morris hepatoma membrane proteins on the left of each gel and with liver membrane proteins on the right, are shown in Fig. 3. More than 15 bands are resolved in these electrophoretic conditions (pH 4.5) for either the liver or the tumor. Two slowly migrating components that are present in the liver membrane extract (arrows) seem to be absent in the tumor half-gel. A difference between microsomal smooth membrane proteins from liver and from Morris hepatoma has also been found by coelectrophoresis of dually labeled proteins (Chart 1). Membrane proteins from liver were labeled with $^3$H and those from hepatoma were labeled with $^{14}$C. As shown in Chart 1, the liver pattern contains 2 additional peaks at the top of the
Smooth Membrane Protein in Liver and Hepatoma

Fig. 3. Duplicate samples of split gels loaded with membrane proteins from Morris hepatoma (left) and normal rat liver (right). Electrophoresis was carried out at pH 4.5 in 8 M urea-containing gels; the gels were stained with 1% Amido black and destained electrophoretically.

Fig. 4. Duplicate samples of split gels loaded with membrane proteins from liver (right) and kidney (left) of Buffalo rats. Electrophoretic conditions are the same indicated in the legend to Fig. 3.

DISCUSSION

The 1st question is of the significance of the absence of the 2 protein bands in hepatoma membranes. As an approach to this problem, a comparison has been made between the electrophoretic patterns of microsomal smooth membrane proteins of 2 kinds of cells that are well differentiated toward diverse functions and that were expected to differ widely in their membrane composition. Fig. 4 shows the comparison of liver and kidney smooth cell membranes carried out by the split-gel technique. There is a similarity of the overall patterns with a few major components peculiar to each membrane or represented in different relative amounts. Two distinctly differentiated cells do not differ between themselves by more than a few components when compared on acrylamide gels. This indicates that a change of the electrophoretic pattern, although restricted to few components as in the case of the examined hepatoma, may be significant for the properties of cell membrane. Unfortunately, at present it is hard to discuss the meaning of the changes in protein composition between the liver and a well-differentiated hepatoma, as nothing is known about the existing correlations between the control of cell growth and the structure of cell membranes. It is generally believed, however, that changes at the level of surface cell membranes may play an important role in conferring to the neoplastic cell the peculiar behavior of infiltrative growth, metastasis, and uncontrolled replication. On the other hand,
Chart 1. Fractionation on acrylamide gel at pH 4.5 of dually labeled membrane proteins from Morris hepatoma and normal rat liver. Morris hepatoma proteins were labeled with dimethyl sulfate-14C (○), liver membrane proteins with dimethyl sulfate-3H (●). Dually labeled proteins (specific activity, 500 to 1000 cpm/Mg) were subjected to acrylamide electrophoresis; the gels were sectioned and counted dually.

the relationship between alterations at the level of endoplasmic reticulum and neoplastic growth are still unknown. Nevertheless, our finding is consistent with a similar one obtained by comparison of hamster fibroblasts cultured in vitro before and after their transformation with an oncogenic virus (3). In this system, which is more suitable for labeling and isolation of membrane fraction, the missing of membrane components highly labeled by glucosamine has been observed either at the level of surface or endoplasmic reticulum membrane.

The defect in the composition of smooth endoplasmic reticulum membrane, observed in a well-differentiated liver tumor, might represent an aspect of a general defect of the tumor cell in the assembly of cell membrane.

A major and general criticism on comparative studies of protein composition of normal and neoplastic cell membrane arises, on the other hand, from the observation that tumor cell membranes shed glycoproteins more rapidly in the centrifugation medium than do normal cell membranes (1, 3), and the finding presented in this paper might be interpreted in this sense.

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