Isolation and Characterization of Plasma Membranes from Transplantable Human Astrocytoma, Oat Cell Carcinoma, and Melanomas

Aileen F. Knowles,² Jose F. Leis, and Nathan O. Kaplan

Department of Chemistry and the Cancer Center, University of California, San Diego, La Jolla, California 92093

ABSTRACT

Purified plasma membranes were obtained from five transplantable human tumors, a Grade IV astrocytoma, an oat cell carcinoma, and three melanomas. Plasma membrane fractions were isolated from tumor homogenates by differential and discontinuous sucrose gradient centrifugation. Determination of enzyme activities indicated that the plasma membranes were enriched 10- to 20-fold with respect to 5'-nucleotidase, nicotinamide adenine dinucleotide glycohydrolase, Mg²⁺-activated nucleoside triphosphatase, and sialic acid. Specific activities of nearly all the enzymes varied with the individual tumors, even among tumors of the same type, i.e., the melanomas. Electrophotographs of the plasma membrane fractions showed smooth single-membrane vesicles with slight contamination by lysosomes. Therefore, these membranes are suitable for comparative biochemical studies and for the preparation of tumor-specific monoclonal antibodies.

Plasma membranes from all five tumors contained very high Mg²⁺-adenosine triphosphatase (ATPase) activities. The Na⁺-K⁺-ATPase was a minor component of the total ATPase of these membranes (<30%). The major component was an ATPase exhibiting similar activity toward several nucleoside triphosphates. The activity of such a nucleoside triphosphatase has been correlated with tumorigenicity in cultured liver epithelial cells. The nucleoside triphosphatase of the plasma membranes of astrocytoma and oat cell carcinoma was stimulated from 50 to 100% by concanavalin A, whereas ATPase of the melanoma plasma membranes was not or only slightly stimulated. The different response to concanavalin A could be due to differences in the ATPase molecules of the individual tumors or to the different environment of the ATPase.

INTRODUCTION

It is well known that transformation of normal cells into malignant cells is usually accompanied by myriad alterations of the cell surface [see the papers of Hynes (14), Nicolson (25), and Vaheri (33) for review]. That the plasma membrane is the primary site of transformation has been clearly demonstrated recently in the system of Rous sarcoma virus-transformed chicken embryo fibroblasts. In this particular system, a single gene product of the virus, a protein kinase localized in the plasma membranes of the infected cells, appears to be responsible for all the ensuing phenotypic changes (6, 21). Thus, the plasma membranes of malignant cells have become the focal point in the many investigations conducted with the objective of understanding the process of neoplastic transformation. Extensive studies have been carried out on the comparison of cultured virus-transformed versus untransformed cells. In addition to the many compositional changes of the membranes, alteration of transport processes and enzyme activities in the virus-transformed cells has also been noted. However, detailed studies at the molecular level were lacking because of the difficulty of obtaining large amounts of plasma membranes from cultured cells. The supply of tumor material was not a limiting factor in the study of animal tumors where tumors could be induced by carcinogens. Emmelot and Bos (8-10) and Van Hoeven and Emmelot (33) have carried out very thorough studies on the plasma membranes from rat and mouse livers and hepatomas, examining both the chemical composition and the enzyme activities of the membranes. Chatterjee et al. (4) have reported on a comparative biochemical study of the plasma membranes of a series of rat mammary tumors with different metastasizing capacities. The study of the plasma membranes of human tumors has lagged behind mainly because it was impossible to obtain large amounts of tumor material repeatedly from the same source. Large amounts of reproducible tumor materials are necessary in any systematic study for the development of isolation procedures, for the study of labile functions, and for the purification and characterization of the biological molecules of interest. This requirement has been met by the athymic mice because of their ability to tolerate human xenografts. Results of some of our studies on the mitochondrial functions of 3 human tumors have already been published (20). In this paper, we report the isolation and characterization of plasma membranes from 5 transplantable human tumors. Purified tumor plasma membrane fractions not only allow us to examine their properties more critically but can also provide material for preparation of tumor-specific monoclonal antibodies which can be utilized in target-directed chemotherapy.

MATERIALS AND METHODS

Human Tumor Materials. The 5 transplantable human tumors used in this study were a Grade IV astrocytoma (T24), an oat cell carcinoma (T293), and 3 melanomas (T242, T355, and M21). With the exception of M21, the original tumor samples were obtained from patients in the San Diego area. M21 was grown from a cloned human melanoma line which was provided by Dr. R. A. Reisfeld of the Scripps Clinic and Research Foundation. All tumors were maintained by serial transplantation in BALB/nu athymic mice as described by Reid et al. (29). The astrocytomas used in this study were from the 24th to the 49th passage. The oat cell carcinomas were from the 11th to the 19th passage. Melanomas (T242) were from the 25th to 31st passage. Melanomas (T355) were from the fifth to the eighth passage. Melanomas (M21) were from the first to the eighth passage in the athymic mice. The average growth rate of these tumors to a tumor weight of 3
to 10 g/mouse was 3 weeks for astrocytoma, 6 weeks for T355, and 2 to 3 months for M21, T242, and T293.

Isolation of Purified Plasma Membranes and Endoplasmic Reticulum. Usually 30 to 50 g of tumor tissue were used for each preparation. Crude microsomal fractions were obtained by centrifuging the postmitochondrial supernatant at 60,000 x g for 90 min as described previously (20). The further separation of the plasma membrane and endoplasmic reticulum on a discontinuous sucrose gradient was carried out by a modification of the procedure described by Aronson and Touster (1). The microsomal pellet was resuspended in 56% sucrose (w/w) containing 5 mM Tris-Cl, pH 8.0, so that the final sucrose concentration was 43% (w/w) at 1 to 2 ml/g tissue. For T293, 15 ml of the suspension were placed in 25.4 x 89-mm cellulose nitrite tubes. The suspension was overlaid by 10 ml each of 31, 37, and 7% sucrose (w/w) containing 5 mM Tris-Cl, pH 8.0. For T24, T242, T355, and M21, the suspension (10 ml) was overlaid by 6.5 ml each of 34, 31, 27, and 7% sucrose (w/w) containing 5 mM Tris-Cl, pH 8.0. The tubes were centrifuged overnight in a Beckman SW 27 rotor at 26,000 rpm. After centrifugation, the protein bands at the interfaces were collected from the top by Pasteur pipets with tips bent at 90°. The band collected at 43 and 31% sucrose was diluted 2- to 3-fold with ice-cold 0.2% Triton X-100. After 5 min at 37°, the reaction was stopped by adding 2 ml of 0.1 M sodium acetate (pH 5.0), 10 mM p-nitrophenyl phosphate, and 0.1 μCi carrier-free [3H]ouabain was added either 1 μM or 1 mM nonradioactive ouabain. Binding was complete in 30 min at 37°, and the membranes were centrifuged at 100,000 x g for 30 min. The supernatant solution was decanted, and residual solution on the wall of the tubes was removed carefully by Kimwipes. The pellet was then dissolved in 0.2 ml 10% sodium dodecyl sulfate. An aliquot (0.1 ml) was counted with ACS counting solution (Amersham/Searle Corp., Arlington Heights, Ill.).

Electron Microscopy. Membrane fractions for electron microscopy were prepared according to the method of Fleischer and Kervina (11). To 0.5 ml of a suspension containing 0.1 to 0.3 mg membrane protein was added 0.5 ml 5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4. After overnight fixation at 4°, the membranes were pelleted in an SW 50.1 rotor for 15 min at 13,000 rpm. The pellet was washed and then fixed in 1% osmium tetroxide. After removal of osmium tetroxide, the samples were dehydrated in a graded ethanol:water series followed by a graded ethanol:propylene oxide series and embedded in Epon. Thin sections were cut on a Sorvall MT-2 microtome and postfixed in uranyl acetate and lead citrate. Electron micrographs were taken on a Zeiss 10 electron microscope.

Materials. Na-AMP, Tris-ATP, horse heart cytochrome c (type c), ouabain, concanavalin A, p-nitrophenyl phosphate, sialic acid, rotenone, glutaraldehyde, and yeast alcohol dehydrogenase were obtained from Sigma Chemical Co., St. Louis, Mo. Disodium ATP was from Boehringer Mannheim Biochemicals, Indianapolis, Ind. [3H]Ouabain (49 Ci/mmol) was obtained from Amersham/Searle Corp.

RESULTS

Enzyme Profiles of the Purified Tumor Plasma Membranes. The purified plasma membranes obtained from discontinuous sucrose gradient were assayed for an array of enzyme activities including marker enzymes for plasma membranes, i.e., 5'-nucleotidase and Mg2+-ATPase; marker enzymes for endoplasmic reticulum, i.e., rotenone-insensitive NADH-cytochrome c reductase and NAD+ glycohydrolase; and marker enzyme for lysosomes, i.e., acid phosphatase. The activities of these enzymes of the various tumor plasma membranes are listed in Table 1 together with the values obtained for homogenate and purified endoplasmic reticulum for comparison. Table 1 shows that the specific activities of 5'-nucleotidase and Mg2+-ATPase of the plasma of all 5 tumors were 4 to 20 times higher than in the homogenates and were 2.5 to 8 times higher than that found in the endoplasmic reticulum fractions.

Rotenone-insensitive NADH-cytochrome c reductase activity was measurable in the plasma membrane fraction although the specific activities were usually lower than that of the endoplasmic reticulum fraction. Presence of acid phosphatase indicated that the plasma membrane fractions were contaminated with some lysosomes, which were borne out by electron microscopic observations. However, lysosomal contamination could not account for the extremely high activity seen with the plasma membranes of astrocytoma and melanoma (T355).

To our surprise, we found that NAD+ glycohydrolase was a better marker enzyme for the plasma membranes than endo-
Plasma Membranes of Human Tumors

Enzymatic activities and sialic acid content of cellular fractions from 5 transplanted human tumors

Enzyme activities were determined as described under "Materials and Methods." In the assay for NAD* glycohydrolase, residual NAD* was determined by measuring NAD*-cyanide adduct.

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Cellular fraction</th>
<th>5'-Nucleotidase</th>
<th>Mg** ATPase</th>
<th>NAD* glycohydrolase</th>
<th>Rotenone-insensitive NADH-cytochrome c reductase</th>
<th>Acid phosphatase</th>
<th>Sialic acid (μg/mg)</th>
</tr>
</thead>
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<tr>
<td>Astrocytoma (T24)</td>
<td>H</td>
<td>0.025</td>
<td>0.047</td>
<td>0.002</td>
<td>0.017</td>
<td>0.077</td>
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<td></td>
<td>PM</td>
<td>0.750</td>
<td>0.783</td>
<td>0.033</td>
<td>0.058</td>
<td>0.883</td>
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<td>ER</td>
<td>0.108</td>
<td>0.157</td>
<td>0.006</td>
<td>0.112</td>
<td>0.229</td>
<td>4.0</td>
</tr>
<tr>
<td>Oat cell carcinoma (T293)</td>
<td>H</td>
<td>0.013</td>
<td>0.105</td>
<td>0.001</td>
<td>0.018</td>
<td>0.021</td>
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<td>1.526</td>
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<td>0.125</td>
<td>0.113</td>
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<td>0.202</td>
<td>0.094</td>
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<td>0.002</td>
<td>0.025</td>
<td>0.023</td>
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<td>0.141</td>
<td>0.005</td>
<td>0.162</td>
<td>0.119</td>
<td>10.0</td>
</tr>
<tr>
<td>Melanoma (T355)</td>
<td>H</td>
<td>0.012</td>
<td>0.102</td>
<td>0.003</td>
<td>0.019</td>
<td>0.063</td>
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<td>PM</td>
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<td>0.014</td>
<td>0.213</td>
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<tr>
<td>Melanoma (M21)</td>
<td>H</td>
<td>0.086</td>
<td>0.032</td>
<td>0.003</td>
<td>0.004</td>
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<td>PM</td>
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<td>0.164</td>
<td>0.007</td>
<td>0.094</td>
<td>0.077</td>
<td>4.3</td>
</tr>
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</table>

Table 1

Sialic Acid Content of Plasma Membranes. In the determination of sialic acid in the cellular fractions, we found strong absorption in the thiobarbituric acid assay caused by 2-deoxyribose. The chromophore obtained from the plasma membranes shows an absorption maximum at 549 nm which corresponds to that of sialic acid. We believe the shift in absorption maxima is corroborating evidence of the purity of the plasma membrane fractions. After correction for 2-deoxyribose interference, it can be seen that sialic acid was enriched at least 6-fold in the plasma membrane fractions with respect to homogenates (Table 1).

Electron Microscopy of Plasma Membranes. Fig. 1 shows that the best plasma membrane preparations were obtained from astrocytoma (Fig. 1a) and oat cell carcinoma (Fig. 1b) in terms of morphology. The only discernible contaminating organelles were lysosomes. Plasma membrane fractions from the melanomas usually had a more heterogeneous composition (Fig. 1, c to e). In addition to lysosomes, autophagic vesicles were often found in these preparations. However, all the plasma membrane fractions were virtually free of vesicles arising from endoplasmic reticulum. Fig. 1f is an electron micrograph of the endoplasmic reticulum fraction from melanoma (T242). It is characterized by vesicles studded with ribosome particles. Endoplasmic reticulum from the 4 tumors have very similar morphology (not shown).

We have also carried out sodium dodecyl sulfate-polyacrylamide gel electrophoresis on the 5 plasma membrane preparations. The peptide composition of the membranes was very complex with more than 40 discernible bands. Distinct differences between the different tumor plasma membranes could be observed. This was especially pronounced when comparing peptides of lower molecular weights (M.W. <40,000) (data not shown).
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Ouabain Binding to the Plasma Membranes. Another marker enzyme for mammalian plasma membranes is the Na\(^+\)-K\(^+-\)dependent ATPase which is inhibited by ouabain. We found that the ouabain inhibition of the tumor plasma membrane ATPase was quite small as shown in Table 3. However, all the membranes bound \(^{3}H\)ouabain in the range of 20 to 60 pmol/mg protein, which was comparable to values obtained with a plasma membrane fraction from mouse brain, a tissue known to have large amounts of Na\(^+\)-K\(^+-\)dependent ATPase.

Mg\(^2+\)-dependent Nucleoside Triphosphatase Activities of the Tumor Plasma Membranes. Using ATP as the substrate, we found that all the tumor plasma membranes possessed a very active Mg\(^2+\)-activated ATPase. Results in Table 4 showed that other nucleoside triphosphates were also effective as substrates. Activities obtained with AMP were probably derived from 5'-nucleotidase, although values presented in Table 4 were obtained at pH 7.5 whereas 5'-nucleotidase activities (Table 1) were usually measured at pH 9.1. The plasma membranes exhibited little hydrolytic activity toward \(p\)-nitrophenyl phosphate and PP, at neutral pH (data not shown.)

Chart 2 shows that Ca\(^2+\) could substitute for Mg\(^2+\) in activating the ATPase activity. Activities obtained with Mn\(^2+\) were much lower. Although only the data obtained with the plasma membrane ATPase of oat cell carcinoma are presented here, this was true of all other plasma membranes used in this study.

Table 4

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Astrocytoma (T24)</th>
<th>Oat cell carcinoma (T293)</th>
<th>Melanoma (T242)</th>
<th>Melanoma (T355)</th>
<th>Melanoma (M21)</th>
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<tr>
<td>ATP</td>
<td>0.88</td>
<td>1.24</td>
<td>0.56</td>
<td>1.13</td>
<td>0.63</td>
</tr>
<tr>
<td>ADP</td>
<td>0.54</td>
<td>0.26</td>
<td>0.34</td>
<td>0.46</td>
<td>0.33</td>
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<tr>
<td>AMP</td>
<td>0.65</td>
<td>0.20</td>
<td>0.26</td>
<td>0.30</td>
<td>0.77</td>
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<tr>
<td>GTP</td>
<td>0.95</td>
<td>1.20</td>
<td>0.42</td>
<td>0.80</td>
<td>0.47</td>
</tr>
<tr>
<td>IPT</td>
<td>1.07</td>
<td>1.26</td>
<td>0.49</td>
<td>1.11</td>
<td>0.59</td>
</tr>
<tr>
<td>CTP</td>
<td>0.96</td>
<td>1.18</td>
<td>0.44</td>
<td>0.95</td>
<td>0.48</td>
</tr>
<tr>
<td>UTP</td>
<td>0.98</td>
<td>1.28</td>
<td>0.48</td>
<td>0.99</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Effect of Concanavalin A on the Mg\(^{2+}\)-dependent ATPase and 5'-Nucleotidase of Tumor Plasma Membranes. It has been reported that concanavalin A stimulated the plasma membrane ATPase of several types of cells (16, 27, 29). Chart 3 shows that concanavalin A stimulated the plasma membrane ATPases of astrocytoma and oat cell carcinoma considerably, whereas it had little or no effect on the ATPases of the melanoma cell membranes. The concentration of concanavalin A required for stimulation of the ATPase of oat cell carcinoma plasma membranes was one order of magnitude lower than the ones used for stimulation of the ATPase of liver plasma membranes (29). Concanavalin A is also a known inhibitor of 5'-nucleotidase (3). Chart 4 shows that 5'-nucleotidase activity of all 5 tumor plasma membranes was inhibited between 70 and 80% by concanavalin A at 200 \(\mu\)g/ml.

DISCUSSION

The 5 transplantable human tumors used in this study were maintained in the athymic mice. These tumors, in spite of many passages in the athymic mice, retained their histological characteristics and responded to drugs similar to those in human...
In the preparation of plasma membranes, it is necessary to compare tumor tissue and the normal tissue especially in amounts necessary for any isolation work and for study of labile function (e.g., energy-linked functions in mitochondria). However, meaningful comparison can still be made from information obtained with normal and malignant tissue from other species.

The availability of normal human tissues poses a problem, especially in amounts necessary for any isolation work and for study of labile function (e.g., energy-linked functions in mitochondria). However, meaningful comparison can still be made from information obtained with normal and malignant tissue from other species.

This paper deals mainly with the methodology involved in the preparation and characterization of plasma membranes and the best way to determine purity of these membranes. We find it to be easily applicable to other types of tumors.

The preparative procedure described herein was not developed solely for the isolation of plasma membranes. We have also studied mitochondria obtained from these tumors and have avoided using substances, e.g., Zn^{2+} or fluorescein mercuric acetate, which would damage mitochondria. The procedure of Aronson and Touster (1) was followed with 2 modifications. (a) Only the crude microsomal fraction was used as the source of plasma membranes and (b) 2 additional layers of sucrose solution were used in the preparation of plasma membranes from astrocytoma and the 3 melanomas at the step of density gradient centrifugation. In spite of the fact that the 5 tumors were histologically different, this procedure consistently yielded pure plasma membranes.

The purity of these membranes was evaluated by both morphology and the enrichment of the plasma membrane marker enzymes. 5'-Nucleotidase has been found to be exclusively localized in the plasma membranes of HeLa cells (3) and murine ependymoblastoma cells (22) by cytochemical methods. This enzyme was enriched 10-fold in the membrane preparations described above. Similar enrichment was also obtained with respect to Mg^{2+}-ATPase and sialic acid.

We have found that the use of marker enzymes for quantitating the amount of other organelles in the plasma membrane fractions yielded misleading results. This was demonstrated in 3 cases. (a) Cross-contamination of the plasma membrane fraction by endoplasmic reticulum was determined from measurements of NAD^{+} glycohydrolase, a putative marker enzyme for endoplasmic reticulum (15), and the rotenone-insensitive NADH-cytochrome c reductase, a standard marker enzyme for endoplasmic reticulum. Our results indicated that NAD^{+} glycohydrolase was actually a marker enzyme for plasma membranes, in agreement with the finding of Bock et al. (2), who have shown the presence of this activity in liver plasma membranes. (b) Determination of the rotenone-insensitive NADH-cytochrome c reductase indicated that there might be considerable contamination of the plasma membrane fraction with endoplasmic reticulum since the specific activities of this enzyme by the 2 membranes were quite similar. This was not supported by electron-microscopic observation. In consideration of recent reports that electron transport activity has been found in the plasma membranes of liver (13) and Ehrlich ascites tumor cells (19), an inherent electron transport activity could not be excluded from human tumor plasma membranes. (c) Electron micrographs of these membrane fractions showed the presence of a few lysosomes. Determination of acid phosphatase, a marker enzyme for lysosomes, was low in the membranes of 3 tumors (T293, T242, and M21) and high in the plasma membranes of astrocytoma (T24) and melanoma (T355). In the latter instances, the specific activities were similar to or higher than that obtained with purified mouse liver lysosomes. Such high acid phosphatase activities certainly

patients (12). Although we cannot ascertain if the passage in the mice has altered the biochemical composition of the tumor cells, we have found remarkable reproducibility in the mitochondrial functions and enzyme activities of the plasma membranes from these tumors over an extended period of time (2 years). We therefore feel it is a valid system for the comparative study of the biochemistry of human tumors.

In the evaluation of specific alterations in transformed cells, it is necessary to compare tumor tissue and the normal tissue from which it originates. Thus, animal hepatoma has become the best documented system. In the study of human tumors,
could not be accounted for by lysosomal contamination, as
electron micrographs of these membranes showed that lysoso-
mal contamination was very slight. We have also observed a
high phosphatase activity at acid pH’s in intact cells established
in culture from astrocytoma. These preliminary results sug-
gested that, at least in the astrocytoma, there was an active
acid phosphatase on the outer surface of the cells.

Our experience with the use of marker enzymes in evaluating
the purity of membrane fractions has shown that some of the
standard membrane marker enzymes had different distribution
patterns in different cells. Thus, extreme caution needs to be
exercised in interpreting results obtained from marker enzyme
measurements alone.

Among the enzymes we examined, the Mg\textsuperscript{2+}-ATPase
showed much higher specific activity than all the other en-
zymes. This activity was apparently the main contributor to
the total cellular ATPase activity, accounting for the low inhibition
of the ATPase in the tumor homogenates by oligomycin, an
mitochondrial ATPase inhibitor (20). The abundance of this
enzyme in tumor cells was a point of great interest. From its
lack of substance selectivity and its ability to utilize Ca\textsuperscript{2+} as
well as Mg\textsuperscript{2+} as the divalent ion activator, this enzyme was
similar to the cell surface ATPase (17, 30, 31) or CTPase (26)
found on the surface of many different cells. In the 5 tumors
studied, this activity was so strong as to render the evaluation of
the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase difficult, the presence of which was
nevertheless established definitively by the capacity of the
membranes to bind [\textsuperscript{3}H]ouabain (Table 3). The fact that the
Na\textsuperscript{+}-K\textsuperscript{+}-ATPase was overwhelmed by Mg\textsuperscript{2+}-ATPase in
the astrocytoma was striking. Astrocytoma was of glial origin, and
the Na\textsuperscript{+}, K\textsuperscript{+}-ATPase usually accounted for 50% of the total
ATPase activity. We found that ouabain inhibition of the plasma
membrane ATPase prepared from a rat glioma line (C6) was
also 40 to 50% (data not shown) whereas the inhibition of the
plasma membrane ATPase of astrocytoma by ouabain was less
than 20%. In addition to the reduced sensitivity to ouabain,
astrocytoma (T24) also showed a decreased response to the
catecholamines when compared to the C6 cell line. Thus, the
excessive Mg\textsuperscript{2+}-ATPase activity of the astrocytoma might be
of significance in either dedifferentiation or neoplastic trans-
formation.

The role of the cell surface ATPase in the physiology of the
cell has not been established. However, Karasaki and Oikagaki
(17) and Karasaki et al. (18) have correlated the presence of a
cell surface ATPase with high K\textsubscript{m} and V\textsubscript{max} on liver epithelial
cells with oncogenicity. The possibility that the high ATPase
activities in the plasma membranes of the human tumors was
also closely correlated with tumorigenicity should be seriously
considered.

Is the same enzyme molecule responsible for the ATPase
activity in the plasma membranes of all 5 tumors? This question
cannot be answered with certainty. We observed that the plasma
membrane ATPase of astrocytoma and oat cell carci-
noma was stimulated considerably by concanavalin A whereas
the ATPase of the 3 melanomas showed no response (Chart
3). This could not be due to a lack of interaction of the lectin
with the membranes since the 5’-nucleotidase of all 5 mem-
branes was inhibited by concanavalin A (Chart 4). These results
would suggest that either the ATPase molecules themselves
differ or that the membrane environment of the ATPase was
significantly different in the various tumors.

In conclusion, the present study has pointed out the diversity
of the plasma membranes from different tumors and between
tumors of the same type. These were exemplified by (a) the
very low 5’-nucleotidase activity of the oat cell carcinoma
plasma membrane, (b) large amounts of NAD+ pyrophosphat-
ase in the plasma membranes of melanomas (T242), (c) large
amounts of acid phosphatase in the plasma membranes of
astrocytoma and melanoma (T355), and (d) variable response of
the Mg\textsuperscript{2+}-ATPase to concanavalin A. With the purified plasma
membranes from these human tumors available to us, we shall
be able to examine some of the enzymes of interest in greater
detail.

ACKNOWLEDGMENTS

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performing electron microscopy, Neil Talbert for transplanting the tumors, and
Dr. Knowles for help with the subcellular membrane preparations. Dr. Knowles
wishes to thank Dr. George Fortes for suggesting the [\textsuperscript{3}H]ouabain-binding exper-
nents.

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Fig. 1. Electron micrographs of tumor plasma membrane and endoplasmic reticulum fractions. Plasma membranes were from: astrocytoma (a); oat cell carcinoma (b); melanoma (T242) (c); melanoma (T355) (d); melanoma (M21) (e) and endoplasmic reticulum from melanoma (T242) (f). × 25,700.
Isolation and Characterization of Plasma Membranes from Transplantable Human Astrocytoma, Oat Cell Carcinoma, and Melanomas

Aileen F. Knowles, Jose F. Leis and Nathan O. Kaplan


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