**Mitochondrial Membrane-associated Properties of Morris Hepatomas**


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**SUMMARY**

The distribution of mitochondrial marker enzymes in subcellular fractions isolated by differential centrifugation was determined in Morris hepatomas 21, R3B, and 7794A, and in host and normal livers. Hepatoma 7794A showed increased amounts of mitochondrial enzyme activity in a subcellular fraction requiring higher g forces for sedimentation, while hepatomas 21 and R3B contained increased mitochondrial enzyme activity in a subcellular fraction sedimenting at lower g forces than normally required. It is concluded that these hepatomas contain a greater proportion of mitochondria that differ in size (and/or density) from those of host or normal liver. In the case of Morris hepatoma 21, electron microscopy of tumor tissue also provided *in situ* evidence for changes in mitochondrial size. The ability of mitochondria from hepatoma 21 sedimenting in the nuclear and mitochondrial fractions to carry out oxidative phosphorylation was examined, and both fractions were shown to be relatively tightly coupled; uncoupling was observed upon addition of carbonyl cyanide- m-chlorophenyl hydrazone. Electron micrographs showed typical conformations for both States 3 and 4 respiration. However, when changes in fluorescence of the probe 8-anilino-1-naphthalene sulfonic acid were studied, it was shown that the addition of carbonyl cyanide-m-chlorophenyl hydrazone to energized mitochondria from Morris hepatoma 21 and 16 did not result in an increase in fluorescence as observed for normal or host liver preparations. Differences due to inner-membrane matrix changes were not noted for the hepatomas when Lubrol WX was used to initiate swelling of mitochondria. These latter observations are interpreted to reflect organizational differences at the membrane level for Morris hepatoma 21 and 16 mitochondria.

**INTRODUCTION**

In a previous study of the slow-growing, well-differentiated Morris hepatoma 16 (4), the distribution of mitochondrial marker enzymes was found to be associated to a greater extent with a subcellular fraction requiring higher g forces than normal for sedimentation. Thus, the usual distribution pattern obtained for normal or host liver preparations was not obtained for this tumor. Furthermore, in isolated subcellular fractions and tissue sections, hepatoma mitochondria appeared smaller than those of host liver (or normal liver). Thus, results from both electron microscopy and the enzyme distribution studies indicated the presence in Morris hepatoma 16 of an increased proportion of mitochondria smaller in size than normal.

Other investigators have observed that many fast-growing, poorly differentiated hepatomas contain mitochondria that are smaller in size and require higher g forces than normal for sedimentation (6, 13). It was thus of interest to assess whether differences in the distribution pattern for mitochondria occurred in other well- and highly differentiated hepatomas. Morris hepatomas 21 (highly differentiated), R3B, and 7794A (both well-differentiated) were examined. Results indicate that the mitochondrial enzymes from these hepatomas also are distributed among subcellular fractions in a manner different from that of host or normal liver. However, in addition to finding increased amounts of mitochondrial enzyme activities associated with a subcellular fraction requiring higher g forces for sedimentation (7794A), as found for Morris hepatoma 16, 2 of the hepatomas, 21 and R3B, contained a greater proportion of mitochondria which sedimented at lower g forces.

Determination of the RCR4 for the various mitochondria-containing fractions from hepatoma 21 indicated fairly tight coupling of oxidation to phosphorylation, compared with the host or normal liver preparations. However, when the extent of change of inner membrane-matrix conformation due to swelling agents, and changes in relative fluorescence of the anionic probe ANS under energizing-deenergizing conditions, were studied with the use of the Mt fraction from hepatomas 21 and 16, differences between hepatoma and host liver mitochondria were apparent. These latter results suggest differences at the membrane level between mitochondria isolated from the well- (or highly) differentiated Morris hepatomas and host or normal liver.

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4 The abbreviations used are: RCR, respiratory control ratio; ANS, 8-anilino-1-naphthalene sulfonic acid; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid; INT, 2-(p-iodophenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride; BSA, bovine serum albumin; CCP, carbonyl cyanide-m-chlorophenyl hydrazone.
MATERIALS AND METHODS

Chemicals. HEPES, INT, sodium succinate, Tris-succinate, Tris-HCl, ADP, BSA (Fraction V), rotenone, and CCP were purchased from Sigma Chemical Co. (St. Louis, Mo.). Lubrol WX was a gift from ICI American, Inc. (Stamford, Conn.), ascorbic acid was purchased from Mallinkrodt Chemical Works (St. Louis, Mo.), and ANS (magnesium salt) was from Nutritional Biochemicals (Cleveland, Ohio). All other chemicals were of reagent grade.

Procedures. Male Buffalo rats bearing Morris hepatoma 21 (Generations 5 and 6), hepatoma R3B (Generation 26), hepatoma 7794A (Generation 39), and hepatoma 16 (Generation 7) were obtained from Dr. H. P. Morris. Procedures concerning tumor-bearing animals, normal animals, homogenization, subcellular fractionation, histology, and electron microscopy have been described previously (4). Homogenates, unless otherwise indicated, were prepared in 250 mM sucrose, 10 mM HEPES, and 1 mM EDTA, pH 7.4, and separated by differential centrifugation into nuclear (N), mitochondrial (Mt), intermediate (I), microsomal (Mc), and cytosol (C) fractions. Mitochondrial enzymes assayed included cytochrome oxidase and succinic dehydrogenase (inner membrane), monoamine oxidase (outer membrane), and ornithine aminotransferase (matrix).

Determination of cytochrome oxidase, monoamine oxidase, and ornithine aminotransferase activity and of protein content was performed as previously indicated (4). Specific activities of enzymes are expressed as follows: for cytochrome oxidase, nmoles O2 per min per mg protein; for monoamine oxidase, nmoles benzaldehyde formed per min per mg protein; and for ornithine aminotransferase, nmoles pyrrole-5'-carboxylate formed per hr per mg protein.

Succinic dehydrogenase activity was measured colorimetrically by the method described by Pennington (15). A mixture consisting of 50 mM potassium phosphate buffer, pH 7.4; 0.1% INT; 50 mM sodium succinate (omitted for blanks); and 250 mM sucrose in a volume of 1.0 ml was prepared. The maximal change in absorbance and the amount of spontaneous contraction were observed by following the changes in absorbance at 520 nm for 6 to 8 min.

Changes in the relative fluorescence of ANS upon energizing-deenergizing mitochondria were carried out essentially as described by Azzi et al. (1). Mitochondria (approximately 0.3 mg/ml) were incubated for 5 min at room temperature in the presence of 250 mM sucrose-20 mM HEPES, pH 7.4; 3 μM rotenone; and 12 μM ANS. Succinate (1 mM) then was added, and the fluorescence was recorded for approximately 1 min when 0.3 μM CCP was added; then fluorescence was recorded for an additional 2 min. Fluorescence was measured with a Hitachi-Perkin Elmer MPF-3 spectrophotofluorimeter. Excitation was at 366 nm and emission was at 470 nm with a band pass of 5 nm.

RESULTS

Histology. The hepatomas were characterized histologically according to the classification of Reuber (17). Hepatoma 21 appeared highly differentiated, and hepatomas R3B, 7794A, and 16 were well differentiated. Necrosis was focal and minimal in all tumors.

Electron Microscopy. Electron micrographs of hepatoma 21 and host liver tissue are shown in Fig. 1. Both hepatoma 21 and host liver contained mitochondria in the orthodox conformation. However, many of the hepatoma mitochondria were "dumbbell" shaped with a longitudinal diameter greater than that of the host liver mitochondria. Cristae often transversed the entire width of the tumor mitochondria. Mitochondria of hepatoma 21 thus appear larger in size than those normally observed. A comparison of the number of mitochondria contained in hepatoma 21 and host liver tissue can be made from the micrographs. The tumor tissue appears to be very thickly populated with mitochondria, containing a much greater number per cell volume than the host liver.

Subcellular Distribution of Enzymes. Data for the subcellular distribution of enzymes for hepatomas 21, R3B, and 7794A and host liver are shown in Table 1. Although hepatoma and respective host liver preparations always were centrifuged simultaneously, results from the host livers were similar and have been averaged in this table. Normal Buffalo rat liver showed distribution patterns similar to that of host livers. Differences between host liver and hepatomas will be considered significant if values for the hepatomas are greater or less than 2 standard errors from the mean for the host liver.

Hepatoma 21 showed a striking increase in the percent-
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue</th>
<th>Specific activity, homogenate</th>
<th>N</th>
<th>Mt</th>
<th>I</th>
<th>Mc</th>
<th>C</th>
<th>% of recovered activity</th>
<th>Recovery%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome oxidase</td>
<td>HL⁴</td>
<td>137 ± 21³</td>
<td>27.5 ± 5.2</td>
<td>68.1 ± 3.8</td>
<td>4.0 ± 2.2</td>
<td>0.3 ± 0.1</td>
<td>0</td>
<td>82.2 ± 10.2</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>156 (135–178)³</td>
<td>71.7 (70.6–72.8)</td>
<td>26.4 (26.0–26.9)</td>
<td>1.7 (1.1–2.2)</td>
<td>0.2 (0.0–0.3)</td>
<td>0</td>
<td>94.4 (93.8–94.9)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>R3B</td>
<td>100 (99–102)³</td>
<td>40.7 (36.2–45.3)</td>
<td>53.9 (50.6–57.3)</td>
<td>5.2 (3.9–6.5)</td>
<td>0.2 (0.1–0.2)</td>
<td>0</td>
<td>104.6 (103.7–105.4)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>7794A</td>
<td>54 (49–60)³</td>
<td>26.2 (22.5–30.0)</td>
<td>59.7 (53.5–65.9)</td>
<td>14.0 (11.5–16.5)</td>
<td>0.1 (0–0.1)</td>
<td>0</td>
<td>114.0 (100.4–129.5)</td>
<td>85.4 ± 5.9</td>
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<tr>
<td>Succinic dehydrogenase</td>
<td>HL</td>
<td>4.07 ± 0.41</td>
<td>25.0 ± 6.5</td>
<td>68.6 ± 3.8</td>
<td>5.5 ± 3.4</td>
<td>0.8 ± 0.1</td>
<td>0.1 ± 0.04</td>
<td>85.8 ± 5.9</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>R3B</td>
<td>1.97 (1.94–2.0)⁰</td>
<td>40.1 (34.9–45.3)</td>
<td>52.7 (49.7–55.7)</td>
<td>6.7 (4.5–8.9)</td>
<td>0.5 (0.5–0.5)</td>
<td>0</td>
<td>83.8 (78.6–89.1)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>7794A</td>
<td>1.90 (1.88–1.92)⁰</td>
<td>23.9 (19.6–28.2)</td>
<td>57.3 (49.0–65.6)</td>
<td>18.3 (14.4–22.3)</td>
<td>0.4 (0.3–0.4)</td>
<td>0.1 (0.0–0.1)</td>
<td>85.4 (81.3–89.6)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td>Monoamine oxidase</td>
<td>HL</td>
<td>3.52 ± 0.73</td>
<td>26.0 ± 5.1</td>
<td>62.9 ± 4.2</td>
<td>7.0 ± 2.2</td>
<td>4.1 ± 1.1</td>
<td>0</td>
<td>101.3 ± 9.2</td>
<td>85.4 ± 5.9</td>
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<td></td>
<td>21</td>
<td>0.67 (0.60–0.74)⁰</td>
<td>60.6 (60.3–60.8)</td>
<td>33.5 (30.1–36.8)</td>
<td>4.4 (2.9–6.0)</td>
<td>1.5 (0–3.0)</td>
<td>0</td>
<td>117.4 (109.0–125.9)</td>
<td>85.4 ± 5.9</td>
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<tr>
<td></td>
<td>R3B</td>
<td>0.35 (0.33–0.37)⁰</td>
<td>31.0 (26.6–35.5)</td>
<td>57.8 (52.4–63.1)</td>
<td>5.6 (4.9–6.3)</td>
<td>5.6 (4.1–7.2)</td>
<td>0</td>
<td>118.0 (106.6–129.3)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>7794A</td>
<td>4.07 (4.01–4.13)⁰</td>
<td>23.4 (19.1–27.8)</td>
<td>56.4 (50.1–62.8)</td>
<td>18.2 (16.3–20.0)</td>
<td>2.0 (1.9–2.1)</td>
<td>0</td>
<td>90.0 (78.9–101.1)</td>
<td>85.4 ± 5.9</td>
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<tr>
<td>Ornithine aminotransferase</td>
<td>HL</td>
<td>416 ± 216</td>
<td>25.2 ± 3.6</td>
<td>66.4 ± 3.5</td>
<td>3.7 ± 1.6</td>
<td>0.1 ± 0.1</td>
<td>4.6 ± 0.8</td>
<td>80.3 ± 11.8</td>
<td>85.4 ± 5.9</td>
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<td></td>
<td>21</td>
<td>3148 (2828–3413)⁰</td>
<td>72.2 (68.8–75.5)</td>
<td>16.7 (14.8–18.9)</td>
<td>1.1 (0.9–1.3)</td>
<td>0.2 (0.1–0.2)</td>
<td>9.8 (4.6–15.0)</td>
<td>82.7 (78.4–87.0)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>R3B</td>
<td>725 (621–829)⁰</td>
<td>52.3 (48.2–56.5)</td>
<td>40.5 (37.1–44.0)</td>
<td>2.6 (1.8–3.3)</td>
<td>0.2 (0.1–0.2)</td>
<td>4.4 (3.4–4.4)</td>
<td>73.7 (70.3–77.1)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>7794A</td>
<td>34 (30–38)³</td>
<td>33.6 (30.0–37.3)</td>
<td>47.0 (40.3–53.3)</td>
<td>14.8 (11.5–18.0)</td>
<td>0.6 (0.3–0.9)</td>
<td>4.0 (3.5–4.4)</td>
<td>96.0 (92.8–96.0)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td>Protein</td>
<td>HL</td>
<td>127.2 ± 16.9⁴</td>
<td>19.5 ± 1.9</td>
<td>22.9 ± 1.9</td>
<td>8.2 ± 1.5</td>
<td>8.5 ± 0.5</td>
<td>40.9 ± 2.2</td>
<td>93.0 ± 3.6</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>169.8 (166.7–173.0)⁴</td>
<td>42.7 (42.1–43.3)</td>
<td>11.7 (11.2–12.5)</td>
<td>6.0 (5.8–6.3)</td>
<td>5.6 (5.4–5.9)</td>
<td>33.8 (33.2–34.3)</td>
<td>89.2 (87.7–90.7)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>R3B</td>
<td>78.4 (71.5–85.3)⁴</td>
<td>23.4 (21.3–25.4)</td>
<td>14.1 (13.4–14.8)</td>
<td>7.2 (6.5–8.0)</td>
<td>7.7 (7.0–8.4)</td>
<td>47.6 (47.5–47.6)</td>
<td>96.8 (93.9–99.7)</td>
<td>85.4 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>7794A</td>
<td>113.0 (112.1–113.9)⁴</td>
<td>23.4 (23.1–23.6)</td>
<td>15.4 (14.0–16.8)</td>
<td>12.1 (11.1–13.1)</td>
<td>7.8 (7.7–8.0)</td>
<td>41.3 (40.5–42.0)</td>
<td>87.7 (84.5–90.9)</td>
<td>85.4 ± 5.9</td>
</tr>
</tbody>
</table>

*Units for specific activity are indicated in “Materials and Methods.”
Where Σ(N + Mt + I + Mc + C) = 100% (5).
% recovery = 100 × Σ(N + Mt + Mc + C)/homogenate.
*HL, host liver.
*Mean ± S.E. of all host liver preparations (2 for each tumor).
*Average of 2 experiments. Numbers in parentheses, range.
*mg/g tissue.
age of mitochondrial enzyme activity recovered in the N fraction. This increase was more than double that of host liver for the 3 mitochondrial enzymes assayed. Correspondingly, the Mt fraction of the tumor contained less than one-half the percentage of mitochondrial enzyme activities of the Mt fraction of host liver, and this decrease was significant for all enzymes. The percentage of activity of the mitochondrial marker enzymes in the other subcellular fractions (I, Mc, and C) from both host liver and tumor was low. Recovery of protein in hepatoma 21 was significantly increased in the N fraction (double that of the host) and was significantly decreased in the Mt, Mc, and C fractions. The increased protein in the N fraction of this hepatoma, with decreases in many of the other fractions including the cytosol, would support the contention that there was an increased number of unbroken cells (and, subsequently, an increased number of mitochondria) sedimenting in the hepatoma N fraction. However, thick (0.5-μm) Epon-embedded sections of both hepatoma 21 and host liver N fractions showed the presence of no unbroken cells.

Morris hepatoma R3B also showed an increased percentage of mitochondrial enzyme activity in the N fraction and a decreased percentage in the Mt fraction, although not to the extent shown for Morris hepatoma 21. The change was most apparent for ornithine aminotransferase, less marked for cytochrome oxidase and succinic dehydrogenase, and essentially unchanged for monoamine oxidase. Changes in protein distribution also were less marked for hepatoma R3B than for hepatoma 21. A similar distribution of protein (or an increased amount, in the case of the C fraction) in the other hepatoma R3B fractions suggests that the increase in the number of mitochondria in the N fraction was not due to unbroken cells. Thus, results of the distribution studies indicate that a greater proportion of the mitochondria from both Morris hepatoma 21 and R3B are larger in size (and/or more dense) than mitochondria from host liver (or normal liver).

In contrast to the data from hepatomas 21 and R3B, hepatoma 7794A contained significantly increased percentages of mitochondrial enzyme activities in the I fraction. These results are similar to those previously reported for hepatoma 16 (4). The percentage of protein also was significantly increased in the hepatoma I fraction and decreased in the Mt fraction. However, unlike hepatoma 16, which had little mitochondrial enzyme activity in the N fraction (4), the activity of this fraction of hepatoma 7794A was similar to the corresponding host liver fraction. The results from the distribution studies indicate that a greater portion of the mitochondria of hepatoma 7794A are smaller in size (and/or less dense) than those of host (or normal) liver.

The specific activities of many mitochondrial enzymes in the hepatoma homogenates differed from those of host liver. Cytochrome oxidase was significantly decreased in hepatoma 7794A, while succinic dehydrogenase activity was significantly lower in hepatomas R3B and 7794A. Monoamine oxidase activity was markedly decreased in hepatomas 21 and R3B. Ornithine aminotransferase activity was quite variable among the host livers, and it is possible that this enzyme may undergo variation in activity with food intake similar to that seen for tyrosine aminotransferase activity (16). Hepatoma 7794A had very low ornithine aminotransferase activity, while the activity in hepatoma 21 was elevated 15-fold. Although ornithine aminotransferase activity in hepatoma R3B was not elevated with respect to the host livers, the tumor did have elevated activity with respect to the host livers from the hepatoma R3B-bearing animals. In addition to changes in enzyme activities, protein in the homogenate/g tissue was significantly higher for hepatoma 21 and significantly lower for hepatoma R3B than host liver.

The recovery of enzyme activity was 80% or greater in most of the experiments. However, recovery was frequently low for ornithine aminotransferase and high (greater than 100%) for monoamine oxidase.

**Correlation of Enzyme Distribution with Enzyme-specific Activity.** An interesting correlation can be made between mitochondrial marker enzyme distribution and specific activity of monoamine oxidase and ornithine aminotransferase in the homogenates of the tumors. Hepatomas 21 and R3B, characterized by greater recovery of mitochondrial enzyme activity in the N fraction, showed low monoamine oxidase activity and high ornithine aminotransferase activity, compared with host liver. On the other hand, hepatomas 7794A and 16, which showed an increased percentage of mitochondrial enzyme activity in the I fraction, had monoamine oxidase activity equal to or greater than that of host liver, while ornithine aminotransferase activity was less than that of host liver. Similar results were obtained using the specific activities of the mitochondrial fraction or the fractions showing an increased percentage of enzyme activities. The activities obtained for cytochrome oxidase and succinic dehydrogenase did not correlate with changes in mitochondrial sedimentation pattern.

**Membrane-associated Functions.** The ability of mitochondria sedimenting in the N and Mt fractions to carry out oxidative phosphorylation was assessed by determining the RCR. The average RCR for Morris hepatoma 21, with succinate, was slightly lower than values obtained for the host liver fractions (2.72 and 3.44 for the N and Mt fractions of the hepatoma; 2.92 and 5.82 for the N and Mt fraction of host liver). However, both hepatoma fractions were still relatively tightly coupled.

Electron micrographs of mitochondria in the N and Mt fractions of host liver and hepatoma 21 during State 3 and 4 respiration are shown in Fig. 2. Mitochondria in both N and Mt fractions from host liver and hepatoma have a similar condensed conformation during State 3. Samples from the Mt fraction of both host liver and hepatoma fixed after 2 min of incubation in State 4 show changes from State 3 conformation, both exhibiting primarily an orthodox type conformation similar to that reported by Hackenbrock (10) for rat liver mitochondria.

When the mitochondria were isolated, with the additional precautions suggested by Sordahl et al. (19), i.e., the use of sucrose, Tris, EDTA, and BSA for the isolating media, and KCl-Tris media for assay purposes, the RCR of hepatoma 21 Mt fraction was 3.78 in contrast to the 3.44 obtained with...
our procedures. The RCR of the Mt fraction isolated from Morris hepatoma 16 by means of the procedure of Sordahl et al. (19) was the same as for host liver with succinate, i.e., 4.52 versus 4.54 for the hepatoma, and slightly higher with glutamate, i.e., 3.97 versus 4.55 for the hepatoma. The rate of oxygen uptake in State 3 for both hepatoma 16 and 21 Mt fractions was less than for the host liver preparation with glutamate (76.6 versus 64.0 and 78.6 versus 61.5), in agreement with the data of Sordahl et al. (19). With succinate, State 3 oxygen uptake was 160.6 versus 150.1 and 214.5 versus 176. The magnitude of the decrease is, however, much less than reported for other hepatomas (19), and agrees more closely with the results of Pedersen et al. (14), who used Morris hepatomas 9618A and 7800. The rate of oxygen uptake during State 4 was slightly less with both glutamate and succinate for hepatoma 16, compared with host or normal liver preparation, whereas the rate increased slightly for hepatoma 21 with both substrates, and accounts for the slight decrease in RCR values.

The addition of CaCl₂ or CCP to State 4 respiration increased the rate of oxygen uptake, and essentially no difference could be detected among mitochondria isolated from the various sources. However, when changes in fluorescence of the probe ANS were studied, it was shown that, upon the addition of CCP to energized mitochondria, no increase in fluorescence was observed with the mitochondria prepared from hepatomas 21 and 16. Typical results are shown in Chart 1. Normally, as shown for host liver, an increase in fluorescence is obtained upon addition of uncouplers such as CCP.

Differences due to inner-membrane matrix changes for hepatomas 21 and 16 versus host liver mitochondria were noted when Lubrol WX was used to initiate swelling of mitochondria. Lubrol WX, a non-ionic detergent, is commonly used to measure maximal enzyme activity, especially of membrane-associated enzymes (18). To our knowledge, this is the 1st report of the use of this detergent to induce swelling of mitochondria. As shown in Chart 2, the initial rate of change in absorbance of mitochondria from host liver suspended in the KCl-Tris media containing 40 to 80 µg Lubrol WX per mg protein is much faster than for hepatoma 16 or 21. The addition of antimycin A (2.8 µM) had no effect on the rate of change in absorbance. Similar results were obtained when a NH₄Cl solution (133 mM NH₄Cl-20 mM HEPES buffer, pH 7.4) was used in place of the KCl-Tris medium. When sucrose (290 mM sucrose-20 mM HEPES, pH 7.4) was substituted for the monovalent ions, essentially no change in absorbance for normal, host, or hepatoma mitochondria was noted. Electron micrographs of host and hepatoma 21 mitochondria suspended in Lubrol WX-sucrose-HEPES medium are shown in Fig. 2, D and H. There is marked homogeneity in appearance of the mitochondria, which exhibit a condensed and twisted type conformation (10, 12).

In contrast to results obtained with Lubrol WX, a similar magnitude of change in absorbance for liver and tumor mitochondria was found with small-amplitude swelling induced by phosphate. The tumor mitochondria failed, however, to show any spontaneous contraction or recovery of absorbance as was observed with the normal or host liver preparation.
DISCUSSION

Although it previously was reported that many tumors contain smaller mitochondria requiring higher g forces for sedimentation (4, 13), findings from the present experiments suggest that well- and highly differentiated hepatomas contain mitochondria which sediment at either lower or higher g forces than required for host or normal liver preparations. Hepatomas 21 and R3B showed an increased percentage of mitochondrial enzyme activities in subcellular fractions sedimenting at lower g forces, while hepatoma 7794A had a greater than normal percentage of mitochondrial enzyme activities in a fraction requiring higher g forces for sedimentation. The increased percentage of mitochondrial enzyme activity in the I fraction of hepatoma 7794A does not appear to be due to mitochondrial fragmentation, as the average ratio of percentage cytochrome oxidase (inner-membrane enzyme) to percentage monoamine oxidase (outer-membrane enzyme) contained in the I fraction was 0.81.

Electron micrographs of tissue sections revealed size differences between hepatoma 21 and host liver mitochondria. Hepatoma 21 was thickly populated with mitochondria, many of which appeared larger in size and "dumbbell" in shape. This dumbbell shape in liver mitochondria undergoing division has previously been described (3, 21).

Studies by Hruban et al. (11), in which the diameter of mitochondria in thin sections of tissue from Morris hepatoma 21 was measured, indicated an increased size range over that for the hepatocyte, whereas the size range for mitochondria from Morris hepatoma 7794A did not differ appreciably from the hepatocyte. The distribution of mitochondria within the size ranges was not reported.

Thus, on the basis of the electron microscopy and distribution studies, it is concluded that a greater proportion of mitochondria of Morris hepatoma 21 are larger (and perhaps more dense) than mitochondria of host liver (or normal liver). Results from distribution studies indicate that more mitochondria of Morris hepatoma R3B are larger in size and/or more dense, whereas those of hepatoma 7794A are smaller in size and/or less dense than host (or normal) liver mitochondria.

The hepatomas with larger (and/or more dense) mitochondria had high ornithine aminotransferase activity and low monoamine oxidase activity, while hepatomas with smaller (and/or less dense) mitochondria had lower ornithine aminotransferase activity and monoamine oxidase activity equal to or greater than that of host liver. These results suggest a basic difference in the mitochondrial composition of these highly and well-differentiated tumors. Swick et al. (20) have reported that liver ornithine aminotransferase activity may be associated primarily with a population of smaller mitochondria localized in the cells near the central vein. Since the hepatomas have lost the orientation of cells to specific blood vessels that is seen in the liver, it is of interest to compare the distribution of ornithine aminotransferase activity to that of the other mitochondrial enzymes. In host liver, ornithine aminotransferase activity was distributed similarly to other mitochondrial enzymes; thus, although biochemical heterogeneity may be present, it may require more sensitive methods (e.g., density gradient centrifugation) for detection. With hepatomas 21 and R3B, however, an anomalous distribution pattern of ornithine aminotransferase activity could be detected by differential centrifugation. Compared with other mitochondrial enzymes, a greater increase in the relative specific activity of ornithine aminotransferase was observed in the N fraction, implying that in these tumors, this enzyme is associated with larger (and/or more dense) mitochondria. Thus, in contrast to the proposed association of ornithine aminotransferase activity with smaller mitochondria in rat liver, the ornithine aminotransferase activity of highly and well-differentiated hepatomas may be associated with a population of larger (and/or more dense) mitochondria.

Membrane-associated functions of mitochondria isolated from hepatomas 21 and 16 have been compared. In agreement with the findings of others (8, 14, 19), oxidation by mitochondria isolated from well-differentiated hepatomas is fairly tightly coupled to phosphorylation and, in the case of the 2 hepatomas studied, the rate of oxygen uptake (nmoles/mg protein) in State 3 is not markedly more depressed than that observed for host or normal liver preparations. Electron micrographs of hepatoma mitochondria taken during States 3 and 4 respiration indicate the conformation is similar to that observed for liver mitochondria. It was not until the response of mitochondria to ANS in the presence of succinate and CCP was investigated that a difference between hepatoma and host liver mitochondria was noted. Whether the results indicate that the hepatomas bind less ANS when CCP is added (1) or that there are differences in the degree of hydrophobicity of the membranes (2) remains to be elucidated.

Differences in response to Lubrol WX also suggest differences between the hepatoma mitochondria and liver mitochondria. The interesting finding that Lubrol WX, in the presence of isoosmotic concentrations of KCl or NH₄Cl, would induce changes in mitochondrial conformation resulting in rapid changes in absorbance, not observed in the presence of sucrose, suggests differences in permeability between monovalent ions and uncharged sucrose molecules as a result of treatment with the detergent. Feo (8) has reported that mitochondria isolated from AH-130 Yoshida ascites hepatoma, showing fairly good coupling of oxidation to phosphorylation (RCR, 2.28 and 3.69 with succinate and glutamate, respectively) did not undergo small or large amplitude swelling to the same extent as liver mitochondria. Various inducing agents studied included 30 mM sucrose, digitonin, and phosphate. More recently, Feo et al. (9) showed that mitochondria isolated from the AH-130 Yoshida ascites hepatoma were highly sensitive to trypsin, small concentrations inducing an immediate swelling. We have been unable to confirm these results using mitochondria from both Morris hepatoma 21 and 16 (results not reported). Differences observed in this study between mitochondria from Morris hepatomas 21 and 16, and host liver are interpreted to reflect organizational differences at the membrane level.
REFERENCES


Fig. 1. Electron micrographs of tissue from Morris hepatoma 21 (A) and host liver (B). x 5600.
Fig. 2. Electron micrographs of isolated mitochondria from host liver 21 (A to D) and hepatoma 21 (E to H). x 15,500. Mitochondria isolated in sucrose-HEPES buffer, pH 7.4. A, host liver N fraction fixed during State 3; B, host liver Mt fraction fixed during State 3; C, host liver Mt fraction fixed after 2 min of incubation in State 4; D, host liver Mt fraction suspended in sucrose-HEPES, pH 7.4, incubated with 80 μg Lubrol WX per mg protein; E, hepatoma N fraction fixed during State 3; F, hepatoma Mt fraction fixed during State 3; G, hepatoma Mt fraction fixed after 2 min of incubation in State 4; H, hepatoma Mt fraction suspended in sucrose-HEPES, pH 7.4, incubated with 80 μg Lubrol WX per mg protein.
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