Effect of X-irradiation on the Permeability of L-Alpha Hydroxy Acid Oxidase From Mouse Liver Peroxisomes

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Mice were exposed to 800 R whole body X-irradiation at a rate of 15 R per minute. Following exposure there was an increase in the permeability of L-alpha hydroxy acid oxidase from mouse liver peroxisomes, and an increased sensitivity of the peroxisomes to low concentrations of Triton X-100. These results indicate that latency characteristics of peroxisomes from irradiated animals are altered compared to the latency characteristics of peroxisomes from control animals.

INTRODUCTION

Peroxisomes (microbodies), the cytoplasmic organelles originally first described in kidney\(^1\) and then in liver\(^2\), have recently been recognized as ubiquitous structures in animal\(^3\-^5\) and plant cells\(^6\). These particles, limited by a single membrane, contain catalase and several oxidative enzymes\(^7\-^8\). It appears that catalase is the only constant component of peroxisomes derived from different sources, whereas the hydrogen peroxide-generating oxidative enzymes differ in number and specificity\(^9\).

The effects of whole body X-irradiation on enzymes associated with the peroxisomes of animal tissue have not been investigated in detail and are poorly understood. Kleinbergs and Bernstein\(^10\) reported that the activity of L-alpha hydroxy acid oxidase, (EC 1.1.3.) an enzyme associated with the peroxisomes of hepatic and renal tissue, is increased 80-140% in mice following whole body X-irradiation. Similarly, Goldfeder\(^11\) showed that the activity of mouse tumor catalase is increased following irradiation. The possible role of catalase in radiation effects on mammals has been reviewed by Thompson\(^12\).

The purpose of this study is to compare the latency characteristics (lack of enzyme activity due to a direct consequence of association with a structural entity) of mouse liver peroxisomes from irradiated animals to peroxisomes obtained from control animals.

MATERIALS AND METHODS

1. **Animals**: Male Swiss-Webster mice, 25-35 g., were purchased from Rancho de Conejo, Vista, California, and maintained in the University vivarium. Animals were starved at least 12 hrs. before they were killed.

2. **Tissue preparation**: All procedures were performed at 4°C. For a given experiment, livers were excised and homogenized in sucrose (0.25 M) by 5 up/down strokes in

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a smooth walled tissue homogenizer fitted with a Teflon pestle rotated at 500 rpm. The homogenate was then passed through 3 layers of cheesecloth and made 20\% (w/v) with sucrose (0.25 M). The 20\% (w/v) whole homogenate was either diluted to 10\% (w/v) with sucrose (0.25 M) and assayed for enzyme activity, or subjected to the following centrifugation scheme. A 20\% (w/v) whole homogenate was centrifuged at 270 g \times 10 \text{ min.} The resulting nuclear pellet was washed, recentrifuged, and the resulting supernatant combined with the initial supernatant. The combined supernatant was centrifuged at 22,000 g \times 30 \text{ min.}, yielding a sedimented, partially purified peroxisome fraction and a final supernatant. The nuclear and peroxisome fractions were resuspended to the original volume of the 20\% (w/v) homogenate with sucrose (0.25 M) and the final supernatant adjusted to twice the starting volume.

3. **Triton X-100 treatment**: To the peroxisome fraction was added an equal volume of various concentrations of freshly prepared Triton X-100 in sucrose (0.25 M). After 30 min. at 4°C, the treated peroxisome fraction was centrifuged at 22,000 g \times 30 \text{ min.} The volumes of the resulting sediment and supernatant were adjusted to the starting volume of the treated peroxisome fraction and the enzyme activities of each component were determined.

4. **Enzyme assay**: L-alpha hydroxy acid oxidase activity was measured by reduction of 2, 6-dichlorophenolindophenol (DCIP) employing 0.5 M L-lactic acid (Sigma) as substrate[13]. The decreasing absorbancy of DCIP was continuously monitored with a Cary model 15 recording spectrophotometer and 21 \times 10^3 \text{ cm}^2 \cdot \text{mole}^{-1} \text{ was used as the molar extinction coefficient}[14]. The reaction mixture without substrate or without homogenate did not spontaneously reduce DCIP. Similarly, the inclusion of Triton X-100 in the assay in the absence of substrate or homogenate did not affect DCIP reduction. In all enzyme assay procedures, reaction rates were linear with enzymatic concentration and the substrate was not limiting.

5. **Protein determination**: Total protein was determined by the method of Lowry et al.,[15].

6. **Radiation procedure**: The animals were exposed to whole-body X-irradiation with a Westinghouse therapy X-ray unit operated at 250 kVp and 15 mA, with 0.5 mm copper plus 1.0 mm aluminum filtration and emitting a beam having a HVL of 1.6 mm copper. Exposure was performed under conditions of maximum backscatter in a lucite chamber. Exposure rate was determined by a 25 R Victoreen ionization chamber placed inside the container. Mice were irradiated at an exposure rate of 15 R per min., with a total exposure of 800 R.

**RESULTS**

1. **Whole homogenate**: Initial studies were performed using a 10\% (w/v) whole homogenate from control and irradiated mice, 24 and 48 hours post exposure. Table 1 shows the results of these experiments. A 24\% increase in specific activity (per mg of total protein of the 10\% (w/v) homogenate) of L-alpha hydroxy acid oxidase was observed over control levels at 48 hours post exposure. Kinetic studies, using L-lactate as substrate, were performed at the time of assay.

The apparent Km values for control, 24 hour and 48 hour post exposure preparations were 3.9 \times 10^{-2} \text{M}, 3.1 \times 10^{-2} \text{M}, and 3.3 \times 10^{-2} \text{M}, respectively.
Table 1. Specific activity of L-alpha hydroxy acid oxidase of a 10% (w/v) homogenate.

<table>
<thead>
<tr>
<th></th>
<th>Specific activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM/min/mg</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.20 ± 0.67</td>
<td>3</td>
</tr>
<tr>
<td>24 hr post exposure</td>
<td>7.04 ± 0.60</td>
<td>2</td>
</tr>
<tr>
<td>48 hr post exposure</td>
<td>8.93 ± 0.62</td>
<td>2</td>
</tr>
</tbody>
</table>

a) Specific activity defined per mg of total protein of the 10% (w/v) homogenate.

The mean and standard deviation are recorded.

2. Enzyme distribution in subcellular fractions: L-alpha hydroxy acid oxidase activity in the nuclear, partially purified peroxisome fraction, and soluble fractions of control and irradiated mice was determined. As the histogram in Figure 1 reveals, no increase in solubilization of enzyme activity occurred after the animals were irradiated, or any changes in relative distribution.

3. Peroxisome fraction: Both specific and total activities of the peroxisome fraction from control and irradiated animals were determined. These results are presented in Table 2.

The increases in total activity (per 100 μl of a two fold dilution of the peroxisome fraction) above control values of the peroxisome fraction are 15% and 33% for the 24 hour and 48 hour mice, respectively. Corresponding increases of specific activity (per mg of total protein of the peroxisome fraction) are negligible for the 24 hour mice and 19% for the 48 hour animals. The discrepancy between the changes in total and specific activities in control and irradiated mice are attributable to elevated protein content in the peroxisome fraction taken from irradiated mice. Average protein content of the peroxisome fraction of control, 24 hour and 48 hour post exposure mouse was 1.09, 1.29, and 1.21 mg per 0.1 ml of a twofold dilution of the peroxisome fraction.

4. Effects of Triton X-100 on enzyme activity of the peroxisome fraction: The per-

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Fig. 1. Subcellular distribution profile of L-alpha hydroxy acid oxidase activity of control, 24 hour and 48 hour post exposure mice. Percentage total activity equals the enzyme activity present in each fraction expressed as the percentage of the activity found in the whole homogenate. Each distribution profile represents an average of three determinations, each determination done on a liver from a single animal.
oxisome fraction of control and of irradiated mice was treated with various concentrations of Triton X-100 and subsequently assayed for enzyme activity. These data are presented in Table 3.

Significant increases in specific activity (per mg of total protein of the peroxisome fraction) were observed after treatment of the peroxisome fraction from control and irradiated mice with Triton X-100 concentrations greater than 0.06%. In the control preparation, a 149% increase in enzyme activity is demonstrated after treatment with 0.25% Triton X-100. The 24 hour and 48 hour post exposure peroxisome fractions showed 102% and 77% increases, respectively.

Figure 2 shows the data from Table 3 as percent of total activity for control, 24 hour and 48 hour peroxisome fractions. Total activity is taken as the specific activity at 0.25% Triton X-100 for each group.

Of prime interest is the percent of total activity detectable with no detergent treatment. The non-irradiated control showed 40.3% of total activity, whereas 24 hour and 48 hour mice initially showed 48.5% and 56.5% of total activity, respectively.

5. **Solubilization of enzyme activity from the peroxisome fraction with Triton X-100**: Release of L-alpha hydroxy acid oxidase activity into the non-sedimenting soluble component from the peroxisome fraction by varying concentrations of Triton X-100 was investigated. Control peroxisome fractions and 24 and 48 hour post irradiation peroxisome fractions were treated with Triton X-100 and centrifuged at 22,000 g×30 min. The soluble activities of all fractions were assayed for enzyme activity.

The data are shown in Figure 3. Total soluble activity (100%) is taken as the soluble activity at 0.25% Triton X-100. At 0.25% Triton X-100 concentration, 92% of the total activity from the peroxisome fraction was solubilized in all cases. Recovery of enzyme activity in the sedimenting and soluble components after Triton X-100 solubilization was always about 90% of the total activity in the peroxisome fraction.

This illustrates that at comparable concentrations of Triton X-100, more activity is released from the peroxisome fraction of irradiated mice than from the peroxisome fraction of non-irradiated control mice. At 0.03% Triton X-100, 1.7% of total soluble

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**Table 2. Total and specific activities of L-alpha hydroxy acid oxidase of the peroxisome fraction.**

<table>
<thead>
<tr>
<th></th>
<th>activity total&lt;sup&gt;a)&lt;/sup&gt; μM/min/100μl</th>
<th>specific&lt;sup&gt;b)&lt;/sup&gt; μM/min/mg</th>
<th>number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>8.49 ± 0.06</td>
<td>7.81 ± 0.05</td>
<td>6</td>
</tr>
<tr>
<td>24 hr post exposure</td>
<td>9.76 ± 0.21</td>
<td>7.53 ± 0.71</td>
<td>6</td>
</tr>
<tr>
<td>48 hr post exposure</td>
<td>11.31 ± 0.00</td>
<td>9.32 ± 0.34</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a)</sup> Total activity defined per 100 μl sample of a twofold dilution of the peroxisome fraction.

<sup>b)</sup> Specific activity defined per mg of total protein of the peroxisome fraction.

The mean and standard deviation are recorded.

**Table 3. Effects of Triton X-100 on the peroxisome fraction.**

<table>
<thead>
<tr>
<th>Triton X-100 concentration-%</th>
<th>specific activity&lt;sup&gt;a)&lt;/sup&gt; μM/min/mg</th>
<th>control</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.81 ± 0.05</td>
<td>7.53 ± 0.71</td>
<td>9.31 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>5.34 ± 0.32</td>
<td>5.95 ± 0.07</td>
<td>8.11 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>0.06</td>
<td>11.35 ± 1.46</td>
<td>8.46 ± 0.73</td>
<td>9.10 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>11.47 ± 1.69</td>
<td>9.73 ± 0.30</td>
<td>9.10 ± 1.74</td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>12.57 ± 1.72</td>
<td>10.16 ± 0.52</td>
<td>9.77 ± 0.53</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>19.35 ± 3.16</td>
<td>15.23 ± 0.49</td>
<td>16.45 ± 0.54</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a)</sup> Specific activity defined per mg of total protein of the peroxisome fraction.

Numbers of mice used were as specified in Table 2. The mean and standard deviation are recorded.
Fig. 2. Triton X-100 concentration versus L-alpha hydroxy acid oxidase activity of the peroxisome fraction from control, 24 hour and 48 hour post exposure mice. Percent of total activity equals the specific activity at 0.25% Triton X-100 for each group. Numbers of mice used were as specified in Table 2.

Fig. 3. Triton X-100 concentration versus L-alpha hydroxy acid oxidase release from the peroxisome fraction of control, 24 hour and 48 hour post exposure mice. Percent of total soluble activity is expressed as the soluble activity at 0.25% Triton X-100 for each group. At 0.25% Triton X-100 concentration, 92% of the total activity from the peroxisome fraction was solubilized in all cases. Numbers of mice used were as specified in Table 2.

activity is released from the peroxisome fraction of control mice, whereas 6.6% and 7.0% of total soluble activity is released from the peroxisome fractions of 24 hour and
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48 hour mice, respectively. This difference seems to persist until the 0.15% Triton X-100 level.

DISCUSSION

Kleinbergs and Bernstein\(^{10}\) showed an increase in mouse liver L-alpha hydroxy acid oxidase activity after 800 R whole body X-irradiation. The range in the percentage increase of activity over control level was 80-140% and the range in time of maximum increase was 20-72 hours post exposure. In the present study we observed a 24% increase in specific activity above control levels in the whole homogenate after 48 hours post exposure, and a 33% increase in total activity in the peroxisome fraction. The differences observed in the magnitude of the change in enzyme activity are attributed possibly to the different strains of mice used and the varying times the animals were killed after exposure.

Also in agreement with the previous study mentioned were the observations that the distribution of L-alpha hydroxy acid oxidase in the homogenate did not shift appreciably from peroxisome bound to soluble activity. The increases in activity of the peroxisome fraction following irradiation substantiate the idea that post-irradiation increases in activity are traceable to changes in the intact peroxisome itself rather than release of the enzyme from the peroxisome following exposure to X-irradiation. Goldfeder \textit{et al.},\(^{12}\) showed that catalase was released into the cell sap in mouse tumor cells following whole body X-irradiation. Catalase is thought to exist in a totally soluble, unbound state in the matrix of the peroxisome\(^{16}\). If the peroxisome membrane was made more permeable by the effects of whole body X-irradiation, it is not unlikely that catalase could “leak” out of the peroxisome into the cell interior. This could explain the results observed by Goldfeder. Since L-alpha hydroxy acid oxidase is probably a core or membrane bound enzyme\(^{16}\) leakage would not result from this membrane alteration following irradiation and the enzyme distribution would not necessarily be shifted to the soluble phase. The solubilization of peroxisomal enzymes is also greatly affected by experimental conditions, such as the purity of peroxisomal preparations or salts in the medium\(^9\).

The increased activity of L-alpha hydroxy acid oxidase observed 48 hours post exposure may be due to an increased number of peroxisomes as a result of irradiation. We have recently shown a 19% increase in peroxisome number 48 hours after an exposure of 800 R whole body X-irradiation. After an exposure of 1000 R a 48% increase over control levels is noted in peroxisome number 2 days after irradiation (unpublished data). This observation coincides with the increase in protein content shown in the peroxisome fractions obtained from irradiated animals.

Different degrees of latency of the peroxisome fraction were observed in the non-irradiated control preparations and the preparations extracted from irradiated mice. The non-irradiated control peroxisome preparation displays the greatest latency: 40% of total enzyme activity is initially detectable without treatment by Triton X-100. Latency decreases with time following irradiation. In the fraction from 24 hour mice, 48% of total enzyme activity is initially detectable. In the 48 hour preparation, 56% of total enzyme activity was assayed without Triton X-100 disruption of the peroxisome fraction.
The decreased latency in the peroxisome fraction from irradiated mice is interpreted as evidence supporting the theory that the permeability of the peroxisome membrane is altered by the effects of whole body X-irradiation.

Finally, it would seem that the altered permeability of the peroxisome membrane following whole body X-irradiation is accompanied by a measurable increase in the fragility of the peroxisome membrane. Figure 3 showed that peroxisome bound L-alpha hydroxy acid oxidase is solubilized by lower concentrations of Triton X-100 in enzyme preparations taken from 24 hour and 48 hour mice than in those preparations taken from non-irradiated control mice. The effects of whole body X-irradiation appear to lower the resistance of the peroxisome membrane to disruption by detergents. If the observed fragility of the peroxisomes is a result of radiation-induced damage to the membranes or possibly from newly formed fragile peroxisomes is not known. Similar results have been shown to occur in lysosomes after X-irradiation\(^{17}\). The release of acid phosphatase, a latent lysosome enzyme, has been demonstrated in regenerating rat liver following whole body X-irradiation\(^{18}\). Identical results were demonstrated by Rahman\(^{19}\) in rat thymus and spleen. In later work, Rahman\(^{20}\) showed that this release of acid phosphatase from the lysosome was accompanied by an increased fragility of the lysosome membrane. In the absence of detergent (Igepal–630), a difference of not more than 10% was seen in the amount of enzyme released. However, at the lowest concentration of Igepal–630, 0.01%, spleen lysosomes of irradiated animals released 20% more acid phosphatase than did those of their controls. The increased fragility of the spleen lysosomes of the irradiated animals tended to diminish as the concentration of Igepal approached 0.05%, where 100% release of acid phosphatase was observed in control and irradiated animals. The release of other non-latent particle bound enzymes has been cited by Okada\(^{21}\) as additional evidence that membrane permeability is altered by the effects of radiation. In addition to catalase and the lysosome enzymes previously discussed, Okada lists nuclear lactic dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase and mitochondrial adenosine triphosphatase as enzymes solubilized by the effects of whole body X-irradiation. In all cases the release of the enzymes was progressive after irradiation and was not immediate.

Considerable progress has been made in the understanding of the functional role of peroxisomes in plant cells, the importance of these organelles in cellular metabolism in animal tissues remains to be elucidated. Several studies suggest that peroxisomes may play a significant role in a variety of functions such as in gluconeogenesis, in cholesterol and steroid metabolism, and in the detoxification of hydrogen peroxide\(^{22-23}\).

If, in the future, the liver peroxisomes are implicated in gluconeogenesis, the increases in L-alpha hydroxy acid oxidase activity following irradiation could be important in supplying additional carbohydrates for Krebs cycle oxidations and for intermediates of glycolysis. Presumably, repair of radiation-injured cells could be expedited by the carbohydrates indirectly produced by additional L-alpha hydroxy acid oxidase activity.

ACKNOWLEDGMENT

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REFERENCES


