Effect of proteolytic and lipolytic Enzymes on the Electron Transport Particle Fraction of *Rhodospirillum rubrum*

I. Proteases

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Digestion of the electron transport particle fraction of *Rhodospirillum rubrum* with the proteases chymotrypsin, trypsin, subtilisin and pronase resulted in a release of protein from the membranal system. The solubilization was, however, limited to only 15—18 percent of the total protein, being the same with each of the four proteases. The enzymes catalyzing electron transfer which are located on these particles were not solubilized by the proteolytic digestion but were found to become differently inactivated already with low protease concentrations. The proteolytic attack revealed differences in the specificity of proteolysis between chymotrypsin and the other proteases.

Among the numerous methods which have been developed to study the architecture of biological membranes and to elucidate function, reaction mechanisms and localization of the enzymes associated with these membranes are the use of detergents and of lipolytic enzymes.

Previous work in this laboratory has been concerned with effects of a number of detergents on the enzymes catalyzing electron transfer in the cytoplasmatic membrane fraction of the photosynthetic bacterium *Rhodospirillum rubrum*. As an extension of this work the present communication deals with the action of proteolytic enzymes on this bacterial membranal system.

Materials and Methods

Trypsin, subtilisin (a protease from *Bacillus subtilis*) and pronase (a protease from *Streptomyces griseus*) were obtained from Sigma, St. Louis, chymotrypsin was a product of Boehringer, Mannheim. All other biochemicals were obtained from sources described earlier.

Results and Comments

As shown in Table 1 incubation of the electron transport particulate fraction with chymotrypsin resulted in an increasing solubilization of protein...
The action of the proteases on the bacterial membranal system does under none of the experimental conditions employed lead to a solubilization of the electron transfer enzymes located on these particles, but rather the different activities are extensively inactivated during an incubation with the proteases. In order to establish a possible minimal solubilization of the enzymes taking place with the inactivation, electron transport particles with 7.5 μg protease/mg membrane protein were kept at 0 °C to allow solubilization to occur (see time zero in Table 1) but to minimize an inactivation. No enzymatic activity was found in the solubilized protein, even after prolonged action of the protease.

In Table 2 the values of inactivation of the different electron transfer enzymes are summarized. It can be seen that already with low concentrations of protease, i.e. 20 μg/mg membrane protein, and at 20 °C, increasing inactivation is observed. More drastic proteolysis or higher temperatures will cause complete inactivation within a short period of time. For reason that some of the enzymes investigated are not stable when incubated at 25 or 30 °C over a longer period, the experiments were performed at 20 °C.

With all four proteases studied NADH-and succinate dehydrogenase are the least inactivated enzymes. From a consideration of the mode of action of the proteolytic enzymes this is to be expected as each of the two activities comprises

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Table 1. Solubilization of protein from the electron transport particulate fraction by digestion with chymotrypsin. Electron transport particles (4.8 mg protein/ml) were incubated with the stated concentrations of chymotrypsin. A control without protease was included. After the indicated periods of time the mixture was chilled and immediately centrifuged at 105,000 g for 45 minutes. Then the amount of solubilized protein was determined in the supernatant (corrected for the protease protein present). The values are percent solubilized protein.

<table>
<thead>
<tr>
<th>mg membrane protein</th>
<th>Time at 20°C [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0 10 20 30 60</td>
</tr>
<tr>
<td>100</td>
<td>8 12 16 17 16</td>
</tr>
</tbody>
</table>

Table 2. Inactivation of the electron transfer activities with various proteases. Electron transport particles (4.8 mg protein/ml) were incubated with the indicated proteases (20 μg protease protein/mg membrane protein) at 20 °C for 30 and 60 min, respectively. Then the different electron transfer activities were determined as described. Corrections were made with the first three activities for a minimal (not exceeding 5–7 percent) endogenous inactivation after 60 min at 20 °C.

<table>
<thead>
<tr>
<th>Activity</th>
<th>% Inactivation</th>
<th>Chymotrypsin</th>
<th>Trypsin</th>
<th>Subtilisin</th>
<th>Pronase</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH oxidase</td>
<td>27 44 21 25 35 26 38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADH-cytochrome reductase</td>
<td>15 28 40 59 40 65 48 68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate-cytochrome reductase</td>
<td>17 27 53 70 44 70 50 73</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADH dehydrogenase</td>
<td>10 15 15 15 14 14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>16 12 20 19 21 21</td>
<td></td>
<td></td>
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</tbody>
</table>

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only a short segment of the electron transport chain. Accordingly the NADH oxidase system, a measure of the complete chain, is found more sensitive to the action of the different proteases. As can be seen, only minor differences in the inactivation of the above mentioned enzymes exist between the four proteases. In contrast, NADH- and succinate-cytochrome c-reductase are differently affected whether the membranes are digested with chymotrypsin or with one of the other proteases. In the presence of chymotrypsin both electron transfer enzymes are inactivated less than the NADH oxidase system while with each of the other proteases the measured inactivation is significantly higher than that of the NADH oxidase system.

These results demonstrate a different specificity of proteolytic attack of chymotrypsin on one hand and of trypsin, subtilisin and pronase on the other. As the segment of the electron transport chain from NADH or from succinate to cytochrome c which is measured by NADH-and by succinate-cytochrome c-reductase is smaller than the complete chain and so less inactivation would be expected, as it is found with chymotrypsin, it is tempting to speculate that there exists a preferential attack of trypsin, subtilisin and of pronase on the region of the cytochrome c binding site within the electron transfer chain, thus leading to the observed more intensive inactivation. Experiments aimed to protect the cytochrome c binding site by preincubating the particulate fraction with different amounts of cytochrome c before the proteolytic digestion (including incubations with equal amounts of bovine serum albumine as the control) failed to show any slow down of the inactivation of the enzymatic activities as compared with the control. Such protection, on the other hand, is not very likely as electrostatic interactions with phospholipids appear to be the only binding forces of cytochrome c with the electron transport chain.  

The comparatively low percentage of solubilization of the membrane protein that is achieved by proteolytic digestion suggests that a certain amount of protein still remains on the surface of the membranal system. From the ease with which the proteases can attack the enzymes of the electron transport chain it appears that the chain, possibly as an associated enzyme complex, is located on or near the surface of the membranes. However, no decomposition of suggested hydrophobic protein components which would be responsible for the attachment of the complex or its segments to the membran structure is accomplished by the proteolysis. Therefore, only inactivation of the electron transfer enzymes but no concomitant solubilization can occur.

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17 M. Boll, Arch. Mikrobiol. 67, 141 [1969].