Characterization of Minor Subunit of Rice Bran Lipase

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In order to understand the structure-function relationship of enzyme, it is essential to characterize the nature of subunit composing the enzyme molecule. In the previous paper, it was indicated by SDS-polyacrylamide gel electrophoresis that rice bran lipase consisted of subunits held together by disulfide bond(s). The major subunit with a molecular weight of 14,000 has been isolated as yet. Assuming that the enzyme molecule consists of only two major subunits, the calculated molecular weight is rather low in view of the molecular weight 32,000, of the enzyme protein, which was evaluated by SDS-polyacrylamide gel electrophoresis. This predicts the existence of other subunit with a smaller molecular weight. In fact, a peptide with comparable mobility to that of tracking dye was observed in SDS-polyacrylamide gel electrophoresis of lipase after reduction with DTT. Therefore, the present experiments were undertaken to isolate and characterize the minor subunit of rice bran lipase.

Rice bran lipase was purified as described earlier, and was homogenous by polyacrylamide gel disc electrophoresis.

The dissociation of lipase molecule into subunits was attempted by oxidation of disulfide bond(s) between them with performic acid. The lipase (13.2 mg) was dissolved in 1 ml of deionized water. To the lipase solution was added 1 ml of freshly prepared 20% TCA solution and the mixture was allowed to stand for 2 hr at ambient temperature. The resulting precipitate spun down by centrifugation was washed three times with 10% TCA solution. Neither amino acids nor peptides were detected in the recovered supernatant. The precipitated lipase was dissolved in a minimal amount of 30% acetic acid, dialyzed against deionized water and lyophilized. The lyophilized TCA-soluble preparation showed a maximum absorption at 245 nm with faintly discernible shoulder over 280 nm in 30% acetic acid solution. This indicated that the TCA-soluble peptide was likely to be comprised of little aromatic amino acid. The lyophilized TCA-soluble preparation was dissolved in 0.6 ml of 20% TCA solution and the mixture was allowed to stand for 2 hr at ambient temperature. The resulting precipitate was washed with chilled 10% TCA solution. The precipitate was used for isolating the major subunit as reported in the paper. The supernatant obtained through this procedure was freed from TCA with ethyl ether and lyophilized.

The ultraviolet absorption spectrum of this TCA-soluble preparation showed a maximum absorption at 245 nm with faintly discernible shoulder over 280 nm in 30% acetic acid solution. This indicated that the TCA-soluble peptide was likely to be comprised of little aromatic amino acid. The lyophilized TCA-soluble preparation was dissolved in 0.6 ml of 20% acetic acid subjected to a gel filtration through a Bio-Gel P-10 column equilibrated with 20% acetic acid. The column was eluted with the same solution at a flow rate of 3.0 ml per hr. Arrows indicate the elution positions for (a) lysozyme (M.W. 14,000) and (b) oxidized insulin B-chain (M.W. 3400), respectively.

The isolated peptide, i.e., the minor subunit was hydrolyzed at 105°C for 24 hr with 1 ml of 5.7 N HCl containing 0.05% 2-mercaptoethanol. Amino acid analysis of the hydrolysate was performed on a JEOL 6 AH amino acid analyzer. As given in Table I, the amino acid composition indicated that the subunit had an abundance of cysteic acid derived from cysteine or cystine or both, and glycine. Tyrosine and phenyl-
TABLE I. AMINO ACID COMPOSITION OF MINOR SUBUNIT

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Residual number</th>
<th>Amino acid</th>
<th>Residual number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>3</td>
<td>Ala</td>
<td>3</td>
</tr>
<tr>
<td>Thr</td>
<td>1</td>
<td>Val</td>
<td>1</td>
</tr>
<tr>
<td>Ser</td>
<td>5</td>
<td>Leu</td>
<td>2</td>
</tr>
<tr>
<td>Glu</td>
<td>5</td>
<td>Arg</td>
<td>1</td>
</tr>
<tr>
<td>Pro</td>
<td>1</td>
<td>Cys(SO₃H)</td>
<td>7</td>
</tr>
<tr>
<td>Gly</td>
<td>9</td>
<td>Total</td>
<td>38</td>
</tr>
</tbody>
</table>

† Other amino acids not listed were not detected in the amino acid analysis.

† The values were obtained after 24 hr of hydrolysis and calculated on the basis of the assumption that the number of aspartic acid was three.

alanine were negligible in coincidence with the observation on the UV-absorption spectrum of the peptide. On the basis of the amino acid composition, the molecular weight of the minor subunit was evaluated to be about 3900.

With N-terminal amino acid analysis of the minor subunit examined by the PTC-method of Edman, no satisfactory result was obtained. C-Terminal amino acid of the subunit was determined by both hydrazinolysis and carboxypeptidase A digestion. Hydrazinolysis was conducted for 8 hr at 105°C. In the hydrazinolysate, 0.58 mole of glycine and 0.55 mole of phenylalanine per mole of the subunit were detected by the amino acid analysis. Alternatively, 0.98 mole of glycine per mole of the subunit was mainly released by digestion with carboxypeptidase A for 30 min at pH 7.5 and 25°C. However, no phenylalanine was detected under the same conditions. These observations suggested that C-terminal amino acid of the minor subunit was glycine.

The results described above lead to a conclusion that the minor subunit with a molecular weight of 3900 apparently composes the enzyme molecule by linking through disulfide bond(s) with the major subunit having a molecular weight of 14,000, although further work is required to explain how many subunits constitute the entire enzyme molecule.

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REFERENCES