Sir:

Tannase has been known to hydrolyze the ester linkage as well as depside linkage of tannic acid into gallic acid and glucose. The enzyme may be obtained from certain molds of *Aspergillus* and *Penicillium*. The enzyme from the culture broth of *Aspergillus oryzae* has been investigated by Yamada et al.,\(^1\) and some properties of the enzyme were reported.

In the course of investigation on the chemical nature of the active site of tannase, we have found that the enzyme is a typical serine enzyme which contains a serine in the active site of the enzyme. The experimental evidences are briefly presented in this communication.

The enzyme has been purified about 30-fold from the mycelial extract of *Aspergillus flavus* (IFO 5839) grown on the tannic acid medium, by a procedure involving ammonium sulfate and tannic acid precipitations, DEAE-cellulose column chromatography, Sephadex G-200 gel filtrations and acetone fractionations. The final preparation showed a single symmetric peak upon ultracentrifugation and electrophoresis. The molecular weight of the enzyme was calculated to be 192,300 by the approach-to-sedimentation equilibrium method\(^3\) and 194,000 by the sedimentation-diffusion method. The enzyme was a glycoprotein containing 25 per cent of hexose and displayed an isoelectric point at about pH 4.0.

The enzyme was not inhibited by metal chelating agents and SH-reagents but potently inhibited by diisopropylfluorophosphatase (DFP). The inhibition was not immediate, but required a period of preincubation of the enzyme with DFP. The inhibitory effect of DFP on the enzyme was investigated with \(^32\)P-labelled DFP and the results are shown in Table I and Fig. 1. Table I shows the incorporation of radioactivity into the enzyme. The fact that 1 mole of \(^32\)P is incorporated into 1 mole of enzyme to give complete inhibition, is suggestive that the enzyme contains one essential serine per mole of enzyme. The DF\(^32\)P inhibited enzyme was partially hydrolyzed with 2N HCl and the hydrolysate was subjected to the high voltage paper electrophoresis.\(^4\) Radioactive phosphorylserine was identified on the radioautograph of ionogram of the \(^32\)P-labelled enzyme. The radioautograph obtained from the tannase was then compared with those from chymotrypsin and subtilisin. Fig. 1 shows the radioautograph of \(^32\)P-labelled proteins. It is obvious that the amino acid sequence around

\[\text{TABLE I.} \quad \text{\(^32\)P INCORPORATION INTO TANNASE OF *Aspergillus flavus*}\]

<table>
<thead>
<tr>
<th>Incubation time (hours)</th>
<th>cpm</th>
<th>(^{32})P (\times 10^{-9}) mole</th>
<th>(^{32})P/mole enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>89.2</td>
<td>2.21</td>
<td>0.69</td>
</tr>
<tr>
<td>24</td>
<td>107.2</td>
<td>2.65</td>
<td>0.83</td>
</tr>
<tr>
<td>48</td>
<td>98.2</td>
<td>2.43</td>
<td>0.76</td>
</tr>
<tr>
<td>72</td>
<td>115.2</td>
<td>2.85</td>
<td>0.89</td>
</tr>
<tr>
<td>96</td>
<td>125.2</td>
<td>3.09</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The purified enzyme (3.18 \(\times 10^{-9}\) moles) was incubated with DF\(^{32}\)P at the concentration of 3 \(\times 10^{-3}\) M. After incubation, the enzyme was precipitated by the addition of 1.0% tannic acid solution and the precipitate was washed for several times with the tannic acid solution. Radioactivity of the precipitate was counted by a G.M. counter.

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the active serine of the tannase is rather similar to that of subtilisin than that of chymotrypsin.

A detailed account of this work will be described later.

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Five milligrams of enzyme were incubated for 48 hr with DF$_32$P at the concentration of $3 \times 10^{-3}$ M. After incubation, the enzyme was precipitated by the addition of 1.0% tannic acid solution. The precipitate was washed for several times with the tannic acid solution and hydrolyzed with 2 N HCl at 100°C for 20 min. The hydrolysate was dried up in vacuo and dissolved in 0.1 ml of water. An aliquot of the solution was applied on the filter paper (12 x 55 cm) and the ionogram was made under the following conditions: pyridine acetate buffer, pH 3.6 (pyridine:acetic acid:water=1:10:90); at 3500 v, 50 mA; 50 min.

Hideaki YAMADA
Osao ADACHI*
Masahiro WATANABE
Koichi OGATA*

Research Institute for Food Science,
Kyoto University, Kyoto
*Department of Agricultural Chemistry,
Kyoto University, Kyoto

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