COMMUNICATIONS TO THE EDITOR

Binding of Papain to Dialdehyde Starch

INTRODUCTION

Dialdehyde starch (DAS) is a water-insoluble polymeric aldehyde of high molecular weight prepared by periodate oxidation of starch.1 DAS was used by Goldstein et al.2 in synthesizing an insoluble polyfunctional diazotizable resin intermediate for active derivatives of papain in particulate form. We prepared active water-insoluble papain derivatives by the direct reaction of DAS and crosslinked DAS with papain in aqueous solution. Furthermore, we used papain as an inexpensive crude powder in contrast to crystalline papain conventionally used to prepare the immobilized enzyme. Our water-insoluble crosslinked DAS-papain showed the best combination of yield and proteolytic activity of all of the products prepared and accounted for approximately 29% of soluble papain nitrogen and 0.5% of total papain activity. Such low activity has been shown by Brümmcr et al.3 to be characteristic of insoluble bound proteases acting on high molecular weight substrates. However, the crosslinked DAS-papain has the following advantages: 1) Ease of preparation at low cost, 2) good thermal stability and retention of activity, and 3) ready recovery by filtration or centrifugation for repeated use.

EXPERIMENTAL

In a typical preparation, 5 g of DAS of 90% dialdehyde content (Sumstar 190,* Miles Laboratories, Inc., Elkhart, Ind.) was activated by stirring in 45 ml of water at 90°C for 15 min and cooling to 25°C. The slurry was then added to 800 ml of a water solution of 25–50 g of crude powdered papain (Sigma Chemical Co., St. Louis, Mo.) which had been adjusted to pH 7 with 0.1N NaOH. The reaction mixture was diluted to 1 liter with water and stirred slowly at 25°C and pH 7 for 1½ hr. The insoluble product was isolated by centrifugation at 1800 rpm and washed three times by stirring in 800 ml of water with intermediate centrifugation. The reaction cycle was repeated by treating the soluble fraction with a fresh slurry of activated DAS.

Crosslinked DAS was substituted for DAS in one reaction with papain. The crosslinked DAS was prepared by periodate oxidation1 of crosslinked starch (Vulca 90, National Starch and Chemical Corporation, New York, N.Y.).

The insoluble papain semigels were stored under water at 5°C. Portions were removed and used for proteolytic assay. Portions dried at 105°C to determine solids content served for nitrogen assay.

A modification of the casein (Hammersten quality) digestion method of AOAC4 was used in determining the weight ratio (dry basis) of water-insoluble

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papain to soluble papain at equal protease activity. Casein hydrolysis was followed colorimetrically as described by Mehlretter and Weakley.  

RESULTS AND CONCLUSIONS

Table I shows that the reaction of 10 parts of crude papain with 1 part of activated DAS afforded an insoluble product having a dry weight yield of 10% of the added reagents. Reducing the ratio of papain to DAS to 5:1 produced a better yield of insoluble enzyme, but the product had a slightly lower nitrogen content and only one-third the protease activity of the 10:1 preparation. When a ratio of 2:1 was used, the product was nearly inactive; a ratio of 20:1 gave results not much different from those obtained with the 10:1 ratio product.

The amounts of water-insoluble papain derivatives equivalent to 1 mg of free enzyme are given in Table I. The most active derivative possessed 2.5% of the activity of the free enzyme by weight. Proteolytic activity corresponding to 70% of that originally present in the reaction mixture containing 10 parts of papain and 1 part of activated DAS was accounted for in the water-soluble fraction. Repeating the reaction cycle by adding a fresh slurry of activated DAS to the water-soluble fraction produced a second water-insoluble product that had only one-third the activity of the primary product.

Substituting crosslinked DAS for DAS under the same conditions of reaction with 10 parts of papain more than tripled the amount of papain nitrogen that was insolubilized. Furthermore, the insoluble papain was as active enzymatically as the DAS preparation, 43 mg being equivalent to 1 mg of soluble, crude papain, and was obtained in twice the yield. The crosslinked DAS-papain was similar to the DAS preparation in that it had 7% dry substance upon isolation by

<table>
<thead>
<tr>
<th>Weight Ratio of Reactants</th>
<th>Insolubilized Nitrogen, % of Total Nitrogen</th>
<th>Weight Yield, % of Reactants</th>
<th>Nitrogen, % of 1 mg of Papain</th>
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</thead>
<tbody>
<tr>
<td>Papain a</td>
<td>DAS</td>
<td>10</td>
<td>18.9</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>8.4</td>
<td>8.9</td>
</tr>
<tr>
<td>10</td>
<td>1d</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>16.3</td>
<td>7.9</td>
</tr>
<tr>
<td>10</td>
<td>1e</td>
<td>28.8</td>
<td>14.4</td>
</tr>
</tbody>
</table>

a Soluble papain, crude grade; 11.6% nitrogen.
b Stored wet at 5°C; data are calculated to the dry weight basis.
c Digestion of casein at pH 5 and 40°C for 3 hr.
d Nonactivated DAS.
e Crosslinked DAS.
centrifugation at 1800 rpm; however, its semigel structure permitted filtration for isolation and recycling. The high water retention of these insoluble enzymes will allow good penetration of protein substrate into the gels for rapid hydrolysis. Obviously the better overall properties of the water-insoluble enzyme resulting from the substitution of crosslinked DAS for DAS establishes it as the most suitable for practical use.

Before the 10:1 ratio experiments were completed, we evaluated the stability of the protease activity of the 5:1 papain-DAS preparation at 5 and 40°C. Activity was unchanged during storage under water at 5°C for at least 8 months. Figure 1 shows that the protease activity of papain is also thermally stabilized through binding to DAS and changes only slightly during 4 days of incubation at 40°C. In contrast, soluble papain lost 45% of its original activity during the first day and was inactive after 4 days.

The high nitrogen content of the insoluble product from the reaction of 10 parts of papain with 1 part of activated crosslinked DAS indicates that only a small amount of DAS is present. Presumably, intermolecular crosslinking had occurred from the activated dialdehyde groups reacting with some of the functional groups of papain. Solubilization of most of the DAS had taken place through reaction with the crude-grade papain.

When the dialdehyde groups in DAS, which are present predominantly in hemialdal and hemiacetal forms, were not activated, little papain was bound and insolubilized, and the DAS was nearly all recovered by centrifugation. As shown in Table I, the nitrogen content of the water-insoluble product was 0.8%, and DAS content by assay was 88%. The product had only slight enzyme activity, which was probably due to papain absorption.

References


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