ponents combined simultaneously to produce a large unit resembling the native complex. Similar results were obtained previously with Escherichia coli pyruvate dehydrogenase complex (3). The flavoprotein was exchangeable with lipoamide dehydrogenase prepared from two different sources; one of them was independently prepared as a single enzyme by the method described by Massey (4) with some modifications, and the other hydrogenase prepared from two different sources; one of them was prepared from 2-oxoglutarate dehydrogenase complex by chromatography on a gel-cellulose column in the presence of 0.05 M potassium phosphate buffer (pH 7.0). The sedimentation pattern of the reconstituted preparation showed a major peak (Fig. 1) with which the boundary of the yellow color of the flavoprotein was associated. The sedimentation coefficient (65.5 S), the composition, and enzymatic activities of the reconstituted complex were in good agreement with the values of the native complex (Table I). The results indicate that the three isolated components combined simultaneously to produce a large unit resembling the native complex.

Other sedimentation studies indicated that the pyruvate dehydrogenase and lipoamide dehydrogenase did not combine with each other, but each of these components did combine with lipoate acetyltransferase. Similar results were obtained previously with Escherichia coli pyruvate dehydrogenase complex (3). The flavoprotein was exchangeable with lipoamide dehydrogenase prepared from two different sources; one of them was independently prepared as a single enzyme by the method described by Massey (4) with some modifications, and the other was prepared from 2-oxoglutarate dehydrogenase complex by chromatography on a gel-cellulose column in the presence of 2.5 M urea as described by Massey (5). Experiments are in progress to determine the molecular weight of each component and the stoichiometry of the reconstitution of the complex.

**REFERENCES**


**Difference in the Chemical Reactivity of the Disulfide Bonds of Trypsin and Chymotrypsin**

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**SUMMARY**

Sodium borohydride reduces selectively two disulfide bonds in trypsinogen and trypsin which are nonessential for activity. Chymotrypsinogen and chymotrypsin are not reduced under these conditions. The disulfide which is modified in chymotrypsinogen is located in a homologous position in chymotrypsinogen which suggests that the two proteins differ in conformation in this region.

Trypsinogen and α-chymotrypsinogen are considered to be homologous proteins since their structure and properties show striking similarities. The primary structure has related amino acid sequences in all regions and almost half of the amino acid residues are in identical positions (1). Furthermore, four of the six disulfide bonds of trypsinogen (2) are located in the same part of the structure as four of the five disulfide bonds of chymotrypsinogen (3, 4). Finally, it should be noted that trypsin and chymotrypsin, which show differences in specificity, nevertheless appear to have a common mechanism of action (5).

The homologies of sequence and disulfide bridges strongly suggest that trypsinogen and chymotrypsinogen would have many similarities in their tertiary structure. One would then predict that homologous regions would be more alike in conformation and properties than nonhomologous regions. In the present communication, we compare the chemical reactivity of the disulfide bonds of trypsinogen and trypsin with those of chymotrypsinogen and chymotrypsin.

We previously reported that treatment of trypsinogen and trypsin with sodium borohydride resulted in the selective reduction of a limited number of disulfide bonds (6). Experiments described show that two disulfide bonds are accessible in trypsinogen and trypsin but not in either chymotrypsinogen or chymotrypsin. In contrast to predictions, one of the reactive disulfide bonds was identified as a homologous bridge.

The reduction of protein samples was performed in 7-dram vials with 0.1 M NaBH₄ at pH 9.6 and at a protein concentration of 5 mg per ml. The reaction was started by slowly adding an

**TABLE I**

**Sulfhydryl content of chymotrypsinogen and chymotrypsin after reaction with 0.1 M sodium borohydride**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Chymotrypsinogen</th>
<th>Chymotrypsin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.21</td>
<td>0.07</td>
</tr>
<tr>
<td>30</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>45</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>120</td>
<td>0.36</td>
<td>0.29</td>
</tr>
<tr>
<td>180</td>
<td>0.76</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*This work was supported by Research Grant AM09277 from the United States Public Health Service.
†Present address, Bose Research Institute, Calcutta, India.
When the reductive procedure was applied to samples of α-chymotrypsin and chymotrypsinogen, the results were vastly different (Table I). Neither protein was reduced by sodium borohydride under our standard conditions. The small increase in sulfhydryl content was possibly due to a random reduction of disulfide bonds in some of the molecules which became denatured.

We have preliminary evidence at this time that it is one of the homologous cystine bridges which is accessible in trypsinogen but inaccessible in chymotrypsinogen. Partially reduced trypsinogen with two sulfhydryl groups was converted quantitatively to the $^{13}$C-S-carboxymethyl derivative. Complete reduction was then accomplished with mercaptoethanol in 8 M urea, and the newly formed sulfhydryls were converted to nonradioactive S-carboxymethyl derivatives. Characterization of the tryptic digest showed that four radioactive peptides were present. One of these was isolated in 45% yield, and the amino acid composition showed that it agreed with the sequence of trypsinogen from residue 146 to 155. The S-carboxymethyl-cysteine residue at position 184 is identical with half-cystine VII which is linked to half-cystine VIII at position D8 (2). Further work is in progress to identify all of the radioactive peptides and to establish the order in which each of the two accessible disulfides was reduced.

The disulfide bridge, VII-VIII, is located in a homologous region in the structure of trypsinogen and chymotrypsinogen. The difference in chemical reactivity of the cystine bond implies that a difference in conformation exists in trypsinogen and chymotrypsinogen, particularly in the immediate vicinity of the bridge. The location of the disulfide bridge is in a section of the amino acid sequence which Brown and Hartley (4) described as a "methionine loop." Thirteen amino acid residues are within the loop, and only three of the amino acids are identical. It is clear that the nonhomologous amino acids of the loop and others located in close proximity must contribute to the difference in conformation.

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