ENZYMOLOGY OF THE CYCLODEXTRINS

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SUMMARY
The cyclization reaction is a special type of the 4-α-D-glucopyranosyltransfer reactions typical of the cyclodextrin glycosyltransferases (CGTs). The enzyme from K. pneumoniae M 5 simultaneously catalyzes cyclization and an acceptor-dependent transfer of linear fragments of 4-α-D-glucopyranosyl chains (disproportionation). Maximum cyclization rates are obtained with chain lengths of gluc16–gluc80, indicating the dependence upon the helical conformation of the substrate. αCD is formed initially by most of the CGTs. The rates of βCD- and γCD-formation are extremely low. In contrast to αCD the higher CDs scarcely participate in reverse reactions and, therefore, accumulate. Only the incubation time determines which CD is obtained as the main cyclic product. By shifting the reaction equilibrium in the cyclization direction the yields of CDs can be enlarged significantly. Using an ultra-/diafiltration reactor, 70% of starch (enzyme-substrate ratio w/w) 1:100,000) can be converted into CDs.

Essentially 6 bacterial strains (4 bacilli) are known for producing extracellular CGTs. Depending upon the strain and the cultural conditions, 5-430mg of enzyme per 1 of culture filtrate were obtained, and purified up to 140-fold (overall yields 10-78%). Because of the different assay methods the CGTs of the various bacterial sources cannot be compared at present. It cannot be decided, therefore, which enzyme is the most suited for the production of CDs on a technical scale.
The history of cyclodextrin glycosyltransferase research is a very strange one for an enzymologist by several reasons: a) For 70 years only one organism (Bacillus macerans) has been known for producing this type of enzyme. b) Quantitative investigations of the chemistry of the cyclodextrin glycosyltransferases (1,4-α-D-glucan:[1,4-α-D-glucopyranosyl] transferases (cyclizing) (EC 2.4.1.19), CGTs) have been severely handicapped by the lack of a specific assay method. c) As indicated by more than 600 publications and patents (1) the chemists have been fascinated by the clathrate-forming properties of the characteristic cyclic transfer products. In spite of the fact that the CGTs are very interesting enzymes, the investigations of both the enzyme chemistry and the mechanism of the reactions have been neglected.

According to D.French, one of the pioneers in CGT research, "the Schardinger dextrins could be manufactured on a very large scale, if there were a suitable market for them"(2). Up to now, the cyclodextrins (CDs) have been much too expensive for application on a technical scale. Production of CDs of low costs depends upon both an easily available CGT of wellknown characteristics and economic methods for manufacturing the cyclic compounds.

1. Enzyme chemistry. Some molecular characteristics of the CGTs from three bacterial strains are summarized in Table I.

Table I

<table>
<thead>
<tr>
<th>CGT of</th>
<th>Molecular weight</th>
<th>Quaternary structure</th>
</tr>
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<tbody>
<tr>
<td>B.macerans</td>
<td>145,000</td>
<td>dimeric'</td>
</tr>
<tr>
<td>K.pneumoniae M5 al</td>
<td>68,000</td>
<td>monomeric</td>
</tr>
<tr>
<td>alk. Bacillus</td>
<td>88,000</td>
<td>?</td>
</tr>
</tbody>
</table>

Evidently the enzymes from the various bacterial sources differ at least in their sizes and their protein structures. The amino acid analysis of the CGT from Klebsiella pneumoniae M5 al has