Chiral selection and chiral induction by the use of regiospecifically di-(or poly)substituted cyclodextrins

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Abstract - Regiospecific bifunctionalization of \( \beta \)-cyclodextrins is achieved by use of rigid difunctional modifying reagents. Successive (two step) nucleophilic substitution on a regiospecifically capped cyclodextrin leads to a \( \beta \)-cyclodextrin derivative having two different functional groups on regio-specific positions. Such compounds behave as artificial enzymes and receptors. A typical example of an artificial receptor is the cyclodextrin having amino and carboxyl groups on the regio-specific \( \alpha \), \( \alpha \) positions, which recognizes free (unmodified) amino acids quite strongly in a neutral aqueous solution. \( \alpha \)-\( \text{Modified B}_6\)-\( \text{B}([w\text{-amino(ethylamino)}]-\beta\)-Cyclodextrin accelerates conversion from keto acids to L-amino acids in 98/2, 98/2 and 95/5 L/D ratio for phenylglycine, Phe and Trp, respectively.

1. GENERAL STRATEGY FOR ENZYME MIMICKS BY THE USE OF MODIFIED CYCLODEXTRINS

Cyclodextrins, cyclic oligomers (hexamer, heptamer, octamer, etc) of glucose, were easily obtainable from potato starch. Each cyclodextrin has a torus shape cavity inside the molecule, which is surrounded by axial C-H bonds of glucoses, making the environment hydrophobic. From the torus, hydroxyl groups are sticking out, making the molecule water soluble (see Fig. 1).

Most probably water molecules penetrate into the cavity in an aqueous solution, as shown in X-ray crystallography. These water molecules are not satisfactorily hydrogen bonded, in other words they are destabilized to some extent, being readily driven out when a more suitable guest — a hydrophobic guest — comes to contact. Again this situation is well demonstrated by the X-ray crystallography (Fig. 2). The guest best accommodated into the cyclodextrin cavity, therefore, is to be best recognized by the C-H wall of the cavity in a way of best van der Waals surface contact.
The situation still holds for modified cyclodextrins, as typically shown by X-ray crystallography of primary t-BuS-β-cyclodextrin (Fig. 3).

An extremely simple strategy for enzyme mimick is then to be drawn that an efficient catalytic site mimicking an enzyme catalytic site is to be introduced directly onto the "rim" of a certain cyclodextrin torus. A number of examples were reported in literature, which well mimic rather simple enzyme catalysis — enormous acceleration and rather strict substrate specificity of pattern recognition type (size and shape recognition) (ref. 4a, b) (see Fig. 4).

\[
\begin{align*}
(1\text{md})_3 \text{Zn} & ; \text{H}_2\text{CO}_3 \leftrightarrow \text{H}_2\text{O} + \text{CO}_2 & \text{(ref. 5)} \\
(\text{RN})_3 \text{Mn} & ; \text{RCO}_2\text{H} \rightarrow \text{RH} + \text{CO}_2 & \text{(ref. 6)} \\
\text{CO}^+\text{OH} & ; \text{AROCOR} \rightarrow \text{ARO}^- + \text{RCO}_2^- & \text{(ref. 7)} \\
(1\text{md})_2 & ; \text{ArO}^+\text{PO}_2^- \rightarrow \text{ArO}^-\text{PO}_3^- & \text{(ref. 8)} \\
\text{Pyridoxamine} & ; \text{RCOCO}_2^- \rightarrow \text{RCCHCO}_2^- & \text{(ref. 9)}
\end{align*}
\]

2. CHIRAL SELECTION VIA CENTRIPETAL CHIRAL RECOGNITION AIDED BY HYDROPHOBIC BINDING

Guest molecules having hydrophobic group(s) to be strictly recognized by the cyclodextrin cavity often possess hydrophobic binding energy as large as 3 Kcal (ref. 10). Under the circumstances, additional host guest-recognition interaction is operating for other functional groups, X, Y and Z. The situation is well understood by looking from the opposite side of R as shown in Fig. 5b, to which the Newman projection may be appropriately applied. Chiral selection is then to be carried out by the complementary grouping X' Y' and Z' existing along the enzyme wall. A typical intuitive example is selective recognition of hydrophobic l-amino acid derivatives from the d-isomer by α-Chymotrypsin (ChT). The bulky SMe grouping prefers to accommodate the least bulky H and two OH groupings interact with CONH and NHCO via hydrogen bonding.
As a result, d- and l-isomers are bound to ChT with practically the same association constants. Only the difference, significant difference though, between l- and d-isomer binding is the activation of Ser\textsuperscript{195} by His\textsuperscript{57}, but the latter does not exist for the other Ser. This leads to ready hydrolysis of the l-isomer, while d-isomer acts as a competitive inhibitor. In this particular example, X', Y' and Z' define a clockwise direction, which keeps chiral cavity even in the absence of a chiral guest. Whenever a couple of d/l stereoisomers interacts with the host chiral cavity, recognition is achieved via "centripetal" fashion. This chiral selection mode is in a sharp and interesting contrast to the "centrifugal" selection mode often applied to organic synthesis (Fig. 7).

Assuming that this "centripetal" chiral recognition is also important in organic chemistry, one can design a host molecule for chiral selection without much difficulty. A simple-minded example is a host for chiral separation of l- and d-amino acids, an appropriate molecular framework is easily to be drawn after ChT chiral selection mode. Thus, disubstituted cyclodextrins (Fig. 8) are chosen as promising candidates.

A,B- and A,C- capped cyclodextrins were powerful starting materials to reach the synthetic goal (ref. 11). As shown in Fig. 9, straight-forward syntheses provide the target compounds Fig. 8a and b in satisfactory yields (ref. 12).

TABLE 1. Ratio of Disubstituted CD/Mono CD.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Di CD</th>
<th>Mono CD</th>
<th>Di CD/Mono CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>[S–CD]</td>
<td>26</td>
<td>16</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>28</td>
<td>0.423</td>
</tr>
</tbody>
</table>

* [S–CD] = 2.55 × 10\textsuperscript{-3} M

Capping technique (based on "molecular measure" principle) is extremely important for the synthesis of regiospecifically di- (tri-, tetra- etc) substituted cyclodextrins, by which a certain target regiosomer is obtainable much more efficiently and in much better yields than routine, laborious successive substitution (ref. 13) (see Table 1).
TABLE 2. Regioisomer Distribution of Capping Reactions.

<table>
<thead>
<tr>
<th>Capping Reagent</th>
<th>AB</th>
<th>AC</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A,B-(SCH₂CO₂)(NH₄)</td>
<td>97</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>A,B-(Sb)</td>
<td>0</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>A,B-(C₄)</td>
<td>0</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>A,B-(D)</td>
<td>0</td>
<td>8</td>
<td>92</td>
</tr>
</tbody>
</table>

TABLE 3. Chiral Recognition of Tryptophan by Artificial Receptor.

<table>
<thead>
<tr>
<th>CD</th>
<th>Guest</th>
<th>Kₐss</th>
<th>CRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A,B-(SCH₂CO₂)(NH₄)</td>
<td>L-Trp</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>A,B-(Sb)</td>
<td>D-Trp</td>
<td>10.8</td>
<td>0.73</td>
</tr>
<tr>
<td>A,B-(C₄)</td>
<td>L-Trp</td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>A,B-(D)</td>
<td>D-Trp</td>
<td>54.0</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Results of l- and d-amino acid separation in neutral aqueous solution at room temperature are listed in Table 3. Apparently, chiral separation was poor in using the hydrophilic chiral host (Fig. 8a). Two findings are new and worthy to be noted. 1) ΔΗ is reasonably negative but is, in most part, compensated by the accompanying unfavorable entropy change. 2) ΔΗ is much less negative than expected. The latter observation is interpreted by the consideration of very polar local environment, giving very weak polar interaction based on the equation:

\[ U = \frac{Z_1 Z_2}{\epsilon r} \]

The interpretation is verified by the observation of considerably larger negative ΔΗ for chiral host having much more hydrophobic local environment, where d- and l-separation become reasonably efficient. The finding of entropy-enthalpy compensation seems due to loss of rotational freedom of the guest bound to the chiral host. Observed NMR relaxation time confirmed the remaining freedom when a monofunctional guest was bound to a monofunctional CD.

Therefore, an ideal amino acid receptor may be designed as a quadruple recognition type, having a strongly hydrophobic local site.

The present chiral recognition is already reasonably strong, giving a promise of ready d/l amino acid separation in water, when the host is immobilized for column chromatography.

3. CHIRAL INDUCTION VIA CENTRIPETAL CHIRAL RECOGNITION

When a prochiral grouping is fixed in a certain reasonable way in the "centripetal chiral cavity", efficient chiral induction is to be expected. This is the case for aminotransfer reaction in which an amino acid is produced from a keto acid by the help of pyridoxamine. The molecular plane of the pyridoxamine moiety is strictly recognized by the enzyme wall through centripetal interaction as depicted in Fig. 10 for Aspartate aminotransferase (ref. 14). A keto acid, then, is bound to pyridoxamine to give an extended molecular plane still fixed by the centripetal recognition by the enzyme wall, leaving an ω-NH₂ grouping free which is located just above the Schiff-base molecular plane. The prototropy reaction followed, leading to l-amino acid formation. If correct, a very similar situation is gained by use of A,B-disubstituted-β-CD. Thus, B₂-enzyme model, together with its regioisomer (which may be called artificial isozyme) were prepared via straight-forward synthesis (ref. 15) (Fig. 11), but separation of these were not easy at all. A self-selection rule was found powerful in this particular example, which enabled efficient separation of A,B- and B,A-regioisomers. The pyridoxamine regioisomers were converted to the corresponding pyridoxal mixture, to which L-phenylalanine was applied. Interestingly, the A,B isomer was converted back to the pyridoxamine preferentially, while the B,A isomer remained in a form of the pyridoxal. By simple separation of the amine from the aldehyde, the regioisomer separation was successful (see Fig. 12).
Chiral selection and induction by substituted cyclodextrins

Fig. 10. The active site of Aspartate aminotransferase

Fig. 11. The synthetic scheme of \( B_6 \)-enzyme model

Fig. 12. The self-selection of artificial isozymes
TABLE 4. Chiral Induction in Amino Acid Formed Via Artificial B₆ Catalysis.

<table>
<thead>
<tr>
<th>RCO₂H</th>
<th>L/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = CH₂φ</td>
<td>98/2</td>
</tr>
<tr>
<td>= CH₂</td>
<td>95/5</td>
</tr>
<tr>
<td>= φ</td>
<td>98/2</td>
</tr>
</tbody>
</table>

The artificial B₆ enzyme of centripetal chiral recognition capacity then converted several keto acids to the corresponding L-amino acids in 90-96 % ee (Table 4). The B,A-isomer, as expected, provided D-amino acids in similarly selective fashion.

The chiral induction is best interpreted by stereospecific approach of the ω-NH₂ grouping on the B-glucose ring to the B₆-Schiff-base molecular plane which is fixed by covalent bond to the A-glucose ring, hydrophobic binding of an aromatic moiety and hydrophilicity of the CO₂ grouping (Fig. 13).

The mechanism is well supported by the large accelerating effect of the ω-NH₂ grouping (ca. ×10), strong binding of the hydrophobic guests to the cavity and large acceleration therefrom.

REFERENCES