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# MOLECULAR AND CHIRAL RECOGNITION BY CYCLODEXTRINS

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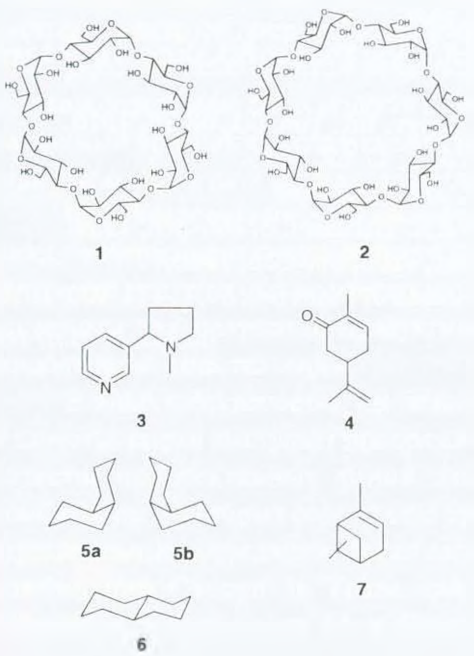
The comparative investigations of the methods of X-ray analysis, NMR, molecular mechanics and molecular dynamics have been carried out for the calculation, prediction and application of the ability of cyclodextrins in molecular and chiral recognition of different enantiomers.

Կատարվել են ռենտգենակառուցվածքային անալիզի, միջուկամագնիսական ռեզոնանսի, մոլեկուլյար մեխանիկայի և դինամիկայի մեթոդների համեմատական հետազոտություններ տարբեր էնանտիոմերների մոլեկուլյար և խիրալ ճանաչման վերաբերյալ ցիկլոդեքստրինների ունակության հաշվարկման, կանխատեսման և կիրառման համար:

Проведено сравнительное исследование методов рентгено-структурного анализа, ЯМР, молекулярной механики и динамики для калькуляции, прогнозирования и использования способности циклодекстринов для молекулярного и хирального узнавания различных энантиомеров.

Native cyclodextrins, CDs, like K-CD **1** and the cheapest and most popular 2-CD **2**, are cyclic oligosaccharides built of 6 or 7 glucopyranoside units, respectively, interconnected by κ-(1, 4) bonds [1]. They are obtained by enzymatic degradation of dextrin. On the basis of X-ray analyses [1b, c], for long time CDs were thought to have a rigid truncated-cone structure with a cavity capable of hosting another molecule, thus selectively forming complexes of different stability [2]. In addition to their significant role as enzyme models [3], their complexes have found several applications in food, cosmetic and pharmaceutical industry, in agrochemistry and other branches [4]. Of special interest is the separation of different or isomeric molecules on the basis of different stability of their cyclodextrin complexes [5].




Chirality is the property of objects that are not identical with their mirror images [6a]. The molecules related to each other as mirror images are called enantiomers. Prevailing majority of drugs and most molecules of which we are built are chiral, therefore drugs typically exhibit enantioselective action. For instance, our organisms

react differently to (*R*)- and (*S*)-nicotine **3** [6]. Similarly, by inhaling (+)- and (-)-carvones **4** we experience the herbaceous, but distinctly different odours; the former is suggestive of dill and caraway seeds while the latter is reminiscent of spearmint [7]. As the consequence of the drugs enantiospecificity, the second enantiomer of a drug (that is usually present as 50% admixture) may have serious side effects [6]. The best known, although not rigorously proved [8], example of such an undesired activity is thalidomide case. In the 70-ties the drug was taken by pregnant women who later bore highly intellingent children with deformities. The use of enantiomerically pure drugs is desirable to avoid side effects of the second enantiomer, thus of particular importance is the CDs application to the separation of enantiomers made possible by their chirality.

**Cyclodextrins structure.**

It should be stressed that CD complexes are very difficult objects for studies since.

1. They form complexes not only with the molecules under investigation but also with impurities. This can pose severe problems when the studied guests have low solubility.

2. They can form complexes of different stoichiometries, ternary complexes involving solvents, etc.

3. The stability constants depend significantly on the experimental conditions such as the solvent, concentration, pH, etc.

4. The CDs size and the specificity of their energy hypersurface characterized by numerous low-lying energy minima cast doubts in the reliability of theoretical results for them and their complexes.

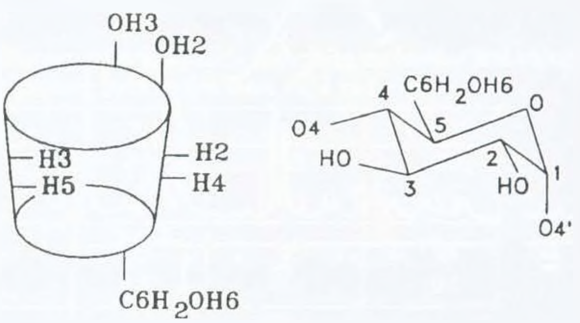



**Fig. 1.** A schematic view of the cyclodextrin spatial structure showing the localization of H3 and H5 protons inside their cavity (left) and the atom numbering in glycopyranoside unit.

As mentioned earlier, on the basis of X-ray analysis for long time CDs were thought to have a rigid truncated-cone structure (Fig. 1) although several experimental and theoretical arguments contradicted this opinion [1d].

1. NMR solution spectra of the complexes involving benzene rings immersed inside the CD cavity consisted of only one signal of H3 (and that of H5) protons [2]. Such a result could not be reconciled with the rigid structure since the so-called ring currents should differentiate the cyclodextrin H3 protons. Similarly, the guest mobility even in the solid state is incompatible with the CDs rigidity [9].

2. Raman spectra and some other experimental results also contradict the CD rigidity [10].

3. The selectivity of the complex formation cannot be understood within the

framework of the rigid CD structure.

4. Both low barrier to internal rotation around glycosidic C-O bond and model calculations for K-CD **2** indicate [11] that these molecules are flexibile. Moreover, the highly symmetrical structures **1**, **2** do not represent the absolute minima but the average structures.

The opinion on the CDs rigidity based on X-ray results was due to the fact that these experimental data were averaged over several molecular positions and long time during which the data were collected. Interestingly, recent, more accurate X-ray results pointed to the CDs flexibility [12].

**Manifestations of chiral recognition by cyclodextrins.**

CDs are chiral and only one CD enantiomer is known. Their interaction with chiral molecules entering their cavity is not identical. This dissimilarity forms the basis for chiral recognition by cyclodextrins. The CD complexes formed with the enantiomers differ in stability and other properties. For instance, bond lengths and angles of two complexes with enantiomeric guests determined by X-ray are not identical [13]. However, the X-ray data are too numerous and complicated to allow one to draw conclusions on the mechanism of chiral recognition by CDs. $^1$H NMR spectra of the complexes exhibit splittings of signals like those shown in Fig. 2 [14]. Much less frequent are reports of the splittings in the carbon spectra (see, however, Ref. 15). NOE effects may also differenciate between the diastereomeric complexes with enantiomeric guest molecules [16].

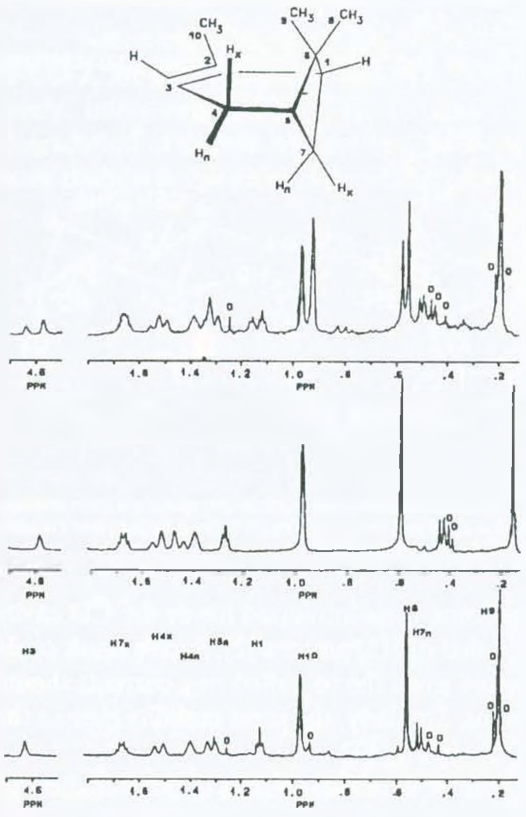


**Fig. 2.** $^1$H spectra in $D_2O$ of (*1S,5S*)- (bottom), (*1R,5R*)-α-pinene (middle), and the racemate (top) with the signal assignment. The signals of impurities are denoted by "o".

**Prediction of molecular and chiral recognition by cyclodextrins on the basis of model calculations.**

In view of practical significance of enantiomer separation by CDs, in particular for chiral drugs, the possibility to predict chiral recognition ability by cyclodextrins would be of great value mainly for pharmaceutical industry.

Hundreds Molecular Mechanic, MM, [17] and few Molecular Dynamic, MD, [18] calculations for the complexes have been published. The calculations have been recently re-

viewed by Lipkowitz [19] who expressed the opinion on the great possibilities of the methods in spite of their sometimes incompetent use. We believe that this opinion is too optimistic on the basis of our calculations of molecular and chiral recognition of decalin isomers by b-CD **2**. At room temperature, *cis*-decalin that forms relatively strong complex with **2** is known to exist as a mixture of two invertomers **5a** and **5b** that are enantiomers as well. When the ring inversion is frozen chiral recognition of the latter enantiomers by **2** manifest itself in $^{13}C$ NMR spectra by splittings of the guest signals [15]. The complex of b-CD with trans-decalin **6** is much weaker [20]. This unique set of experimental data [15, 20 – 21] has allowed us to test the applicability of MM and MD methods to the study of molecular and chiral recognition. The MM calculations [22] have been carried out for four different force fields and five values of electric permittivity e for **6a**, **6b** and **7** and their complexes with **2**. The stabilization energy of the complexes was defined as a difference between the steric energy of a complex and the sum of the energies of its constituent parts. Then, the energy difference between the complexes with enantiomers **6** $DDE_{chir}$ was taken as a measure of chiral recognition while the corresponding difference between the complex with **7** and with the closer in energy complex with either **5a** or that with **5b** $DDE_{mol}$ was considered as a measure of molecular recognition. The results of the calculations are illustrated by the values obtained with the AMBER force field [23] and CVFF force field [24] for e values of 1, 4 and 10 shown in Table 1. (It should be stressed that permittivity is a macroscopic value, thus the choice of this parameter is to a great extent arbitrary). The inspection of the data in the table reveals that:

Table 1. **Dependence of energy differences (in kcal/mol) describing molecular and chiral recognition of decalin isomers by 2 on the force field and e value**

| Force field | DDE | e = 1 | e = 4 | e = 10 |
|---|---|---|---|---|
| AMBER | $DDE_{mol}$ | -0.6 | 1.2 | 0.6 |
| | $DDE_{chir}$ | 1.3 | -0.2 | 1.1 |
| CVFF | $DDE_{mol}$ | 0.5 | 0.6 | 0.3 |
| | $DDE_{chir}$ | 0.8 | -0.4 | 0.3 |

1. Different force fields do not yield the complex with same decalin isomer as the most stable.

2. Contrary to the experimental trends, the $DDE_{chir}$ value characterizing chiral recognition is comparable or even greater than the corresponding $DDE_{mol}$ value characterizing molecular recognition.

This means, that the results obtained by MM calculations are not reliable. This is not surprising since entropy factors, solvent effect, etc. are neglected in such calculations.

MD simulations in vacuum for the complexes of **5a**, **5b** and **6** with **2** [23] revealed that with the AMBER FF [24] they were unstable at 300 K and decomposed during 200 – 250 ps independently on the assumed e value. For the CVFF [25], the complex with the *trans*-decalin is always less stable than those with the *cis*-iso-

mers. However, the sign of $DDE_{clur}$ depends on assumed permittivity value. Therefore, we believe that MD simulations in vacuum can be used only for qualitative studies of molecular recognition.

The experimental data at our disposal did not contain quantitative values of $DDE_{chir}$ for the complexes involving *cis*-decalin enantiomers [15, 26]. Therefore, further studies on the reliability of calculations of chiral recognition by CDs have been carried out in water for the complexes of a-pinene enantiomers **7** with a-CD **1** for which the energy difference between the complexes involving both enantiomers have been published [27]. The first simulations carried out for 3.5 ns yielded excellent agreement with the experimental value of energy difference. the calculations [16]. However, the lengthening of the simulation time to 5 ns yielded small incorrect preference of the complex with (*1R, 5R*) enantiomer of pinene 7 [28].

Further lengthening up to 12 ns reverted this improper trend. It should be stressed that today MD simulations are carried out for few nanoseconds at best. We believe that considerably longer simulations should be carried out to obtain reliable results on chiral recognition by CDs.

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