Formation of Poly(ethylene glycol) Inclusion Complexes in Aqueous Solutions of Mixed Cyclodextrins

*Jiří Horský,** *Zuzana Walterová*

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovského sq. 2, 162 06 Prague 6, Czech Republic

Summary: Even though the addition of modified cyclodextrins (modified CDs) accelerates the precipitation in aqueous solutions of poly(ethylene glycol) (PEG) and α-cyclodextrin (α-CD) the final amount of formed solid complex remains unchanged, with no significant presence of modified CDs detected by MALDI-TOF mass spectrometry. Thus unsuitability of kinetic turbidity measurements for determination of binding parameters was confirmed. On the other hand, theoretical calculations based on a model of a chain of freely accessible binding sites demonstrated that the results do not necessarily contradict the finding that individual modified CD molecules can thread onto PEG chains with the efficiency comparable to that of natural (unmodified) α-CD.

Keywords: cyclodextrins; inclusion complexes; phase separation; supramolecular structures; water-soluble polymers

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides well known for their propensity to form inclusion complexes with various compounds, including polymers. In the latter case, such complexation frequently leads to precipitation and formation of a solid complex. The process is rather sensitive to CD and polymer choice. Modification of CDs mostly interferes with complexation; the cross-sectional diameter of a polymer chain in relation to the size of CD cavity seems to be more important than the polymer chemical composition. For example, poly(ethylene glycol) (PEG) forms inclusion complex with α-cyclodextrin (α-CD) but not with β-cyclodextrin or any modified CD.[1] There is some evidence that threading of individual molecules onto PEG chains occurs even with those CDs that do not form solid complexes with PEG.[2,3] According to turbidimetry, formation of complex in the aqueous solution of α-CD and PEG is accelerated by addition of (2-hydroxypropyl)-α-cyclodextrin (HPACD) which indicates that HPACD participates in formation of a solid complex together with α-CD.[2] Recently, however, the high sensitivity of turbidimetric measurements to experimental arrangement has

DOI: 10.1002/masy.200351327

been demonstrated with cyclodextrins solutions, and the relevance of kinetic turbidimetric measurements for equilibrium properties was questioned.[4] Therefore we investigated the formation of solid PEG complexes in solutions of mixed CDs both theoretically and experimentally.

Results

Effect of Modified CDs on an Overall Yield of the Solid Complex

We determined the amount of solid complex PEG/CDs formed in solutions of PEG (nominal molecular weight 1000) and α-CD, alone or in the mixture with modified CD: HPACD, (2-hydroxypropyl)-β-cyclodextrin (HPBCD), and 2,6-di-*O*-methyl-β-cyclodextrin (DIMEB). The solutions were prepared by mixing the stock solutions of components in appropriate ratios, kept overnight and then centrifuge. The supernatant was removed after another day to ensure that no additional precipitation occurred. The precipitate was weighted immediately after supernatant removal and then after drying under vacuum and net amount of the precipitate was calculated. The results, given in Table 1, contradict the turbidity measurements because no effect of modified CD can be observed: the amount of precipitate corresponds to that in the absence of modified CDs.

Table 1. Formation of solid PEG complex in solutions of mixed CDs.

Exp.No	c_{PEG} 1) mg/mL	$c_{\alpha\text{-CD}}$ 1) mg/mL	c_{HPACD} 1) mg/mL	c_{HPBCD} 1) mg/mL	c_{DIMEB} 1) mg/mL	$m_{precipitate}$ 2) mg
1a	24.0	98.7	---	---	---	82.7
2a	24.0	74.0	---	---	---	54.3
3a	24.0	98.7	24.7	---	---	54.7
4a	24.0	98.7	---	24.7	---	55.6
5a	24.0	98.7	---	---	24.7	55.1
1b	12.0	111.0	---	---	---	84.7
2b	12.0	111.0	12.3	---	---	78.2
3b	12.0	111.0	---	12.3	---	79.6
4b	12.0	111.0	---	---	---	78.6
5b	12.0	111.0	---	---	12.3	74.8

1) Initial concentrations of components

2) Mass of complex in mg, corrected for residual solution, precipitated from 1 mL

Precipitate Composition Determined by MALDI-TOF Mass Spectrometry

The fact that no change in the amount of precipitated complex was observed is not a definitive proof against participation of modified CDs in the solid complex formation because increased complexation could be partially offset by increased solubility of a ternary mixed complex. The unequivocal proof of participation of modified CDs in complexation can be obtained by determining the composition of the solid complex if the ternary solid complex is formed rather than the binary one. For this purpose we used matrix-assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectrometry (MS), a technique especially designed for application of MS to intact macromolecular compounds. In MS spectra obtained from solid complexes prepared as described above, the signals corresponding to modified CDs were very strong. In fact they were so strong that contamination by residual supernatant seemed to be the only adequate explanation. Therefore we repeated the preparation of solid complexes in the same way as before but instead of drying the precipitate we washed it with water and then completely dissolved it. Figure 1 shows MALDI-TOF mass spectrum of precipitate obtained in the presence of HPACD under the experimental conditions corresponding to the experiment number 3a (Table 1). It seems that only traces of HPACD are present in the sample; however, since HPACD is a mixture of compounds with a different degree of substitution it is represented by several peaks in the spectrum which have to be summed to get the total intensity. It turns out that such overall intensity of HPACD is 22% of that of α-CD that is practically corresponding to initial composition of the solution before precipitation. Because MALDI-TOF MS spectral intensity depends both on molecular weight and composition of a compound we carried out measurements of MALDI-TOF MS spectra with various mixtures of α-CD and HPACD which revealed that the α-CD is strongly underrepresented in the spectra. Taking this into account and using the spectra of HPACD/α-CD for calibration purposes, we estimated that concentration of HPACD in washed precipitate is only about 1% of that of α-CD. Moreover, this HPACD need not to be from inclusion complex but can be still from supernatant because thorough washing was precluded by relatively high solubility of the precipitate. Similar results were obtained with HPBCD and DIMEB.

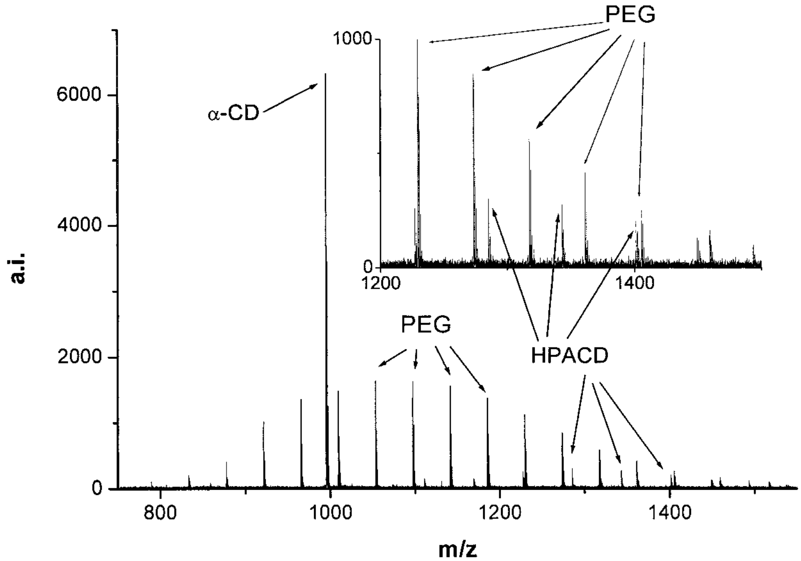

Fig. 1. MALDI-TOF mass spectrum of solid complex precipitated from the solution of PEG, α-CD, and HPACD (see Table 1, experiment No 3a). Bruker Biflex III mass spectrometer was used in a reflectron mode with 2,5-dihydroxy benzoic acid as matrix and NaCl as an ionization agent.

Discussion

The results contradict the turbidity measurements and show that modified CDs are not incorporated into solid inclusion complex of PEG and α-CD. The contradiction can be ascribed to the reported sensitivity of kinetics of α-CD/PEG formation to various factors and can be dismissed as such but the question remains what is the relevance of the current results to the behavior of modified CDs and PEG in dilute solution where threading of individual molecules of modified CDs occurs with the efficiency comparable to that of α-CD.[2] The purpose of turbidity measurements with solutions of mixed CDs was to confirm that modified CDs molecules can indeed form inclusion complexes with PEG even those are soluble ones. The present results show that measurements with concentrated solutions of PEG and mixed CDs give no such confirmation.

In polymer inclusion complexes, only ligands located at polymer chain ends can directly communicate with surrounding solution; the fact, however, has effect only on the kinetics of complexation.[5] The equilibrium properties of a polymer inclusion complex can be described as binding to a chain of independent fully accessible binding sites. Consequently, the partition function for cooperative binding of a ligand A to a linear chain of N binding sites in the presence of non-cooperatively binding ligand B is given as follows

$$\Xi([\mathrm{A}],[\mathrm{B}])=\sum_{a=0}^{N}\sum_{b=0}^{N-a}\sum_{m=0}^{\min(a-1,N-a)\geq 0}\left(\frac{(a-1)!}{m!(a-m-1)!}\right)\left(\frac{(N-a+1)!}{(m+1)!(N-a-m)!}\right)\left(\frac{(N-a)!}{b!(N-a-b)!}\right)(K_\mathrm{A}[\mathrm{A}])^a(K_\mathrm{B}[\mathrm{B}])^b\,h^m$$

K_A, K_B are the binding constants for binding of A, B respectively, and h is the cooperativity coefficient for binding of A. From the partition function the binding isotherm can be calculated. Equilibrium concentrations of ligands, [A] and [B], are not usually known and mass-balance equations for total concentrations must be taken into account. We have calculated the effect of a non-cooperatively binding ligand B on the production of chains fully covered by ligands because it is supposed that α-CD/PEG precipitate is formed by such chains. Addition of the non-cooperative ligand B increases the total amount of fully covered chains (ternary complex) but decreases the amount of chains fully covered by the ligand A only (binary complex). Strong binding of A can suppress the effect of B; strong cooperative binding of A can exclude B from complexation even if values of K_A and K_B are comparable.

If the precipitate is formed the solution concentration of precipitated species is constant which gives an additional requirement to be taken into account in model calculations. Complexation followed by precipitation of the binary complex can easily be analyzed using the theoretical binding isotherm. It turns out that the addition of B that participates in complexation in solution decreases the amount of formed solid binary complex, as long as the solubility of the complex is not too low. The analysis of formation of a ternary solid complex would be more complicated because the existence of equilibrium between solution and a solid complex for each individual species should be considered; however, it is not necessary in the case of modified CDs/α-CD/PEG solutions where only a binary complex precipitates.

Conclusion

Although the addition of modified CDs may accelerate the formation of the solid complex between α-CD and PEG, modified CDs are not incorporated into the solid complex and the final amount of the solid complex remains the same. This finding can be reconcile with the fact that threading onto PEG chains in dilute solutions occurs with the comparable efficiency for both α-CD and modified CDs in two ways: either there are strong cooperative interactions between α-CD molecules adjacent along PEG chain or the solubility of binary α-CD/PEG complex is very low whereas ternary complexes are well soluble. Thus the results obtained with mixed CDs are consistent with both proposed explanation of high yields of α-CD/PEG complexes [1, 4] – strong cooperativity of binding or low solubility of the complex.

Acknowledgement

Financial support by Grant Agency of the Academy of Sciences of the Czech Republic (Grant A1050101) is acknowledged.

[1] A. Harada Adv. *Polym. Sci.***1997**, *133*, 141.
[2] J. Horský *Eur. Polym. J.* **1998**, *34*, 591.
[3] J. Horský *Polym. Bull.* **1998**, *41*, 215.
[4] C. N. Tait, D. M. Davies, *Langmuir* **2002**, *18*, 2453.
[5] J. Horský *Macromol. Theory Simul.* **2000**, *9*, 759