Journal of Inclusion Phenomena and Macrocyclic Chemistry (2005) 53:97–102
DOI 10.1007/s10847-005-1677-8

Binding Delocalization in Polymer Inclusion Complexes of Ring Molecules: Pseudopolyrotaxanes of α-Cyclodextrin and Poly(ethylene glycol)

JIŘÍ HORSKÝ* and BEDŘICH PORSCH
Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague 6, Czech Republic

(Received: 11 November 2003; in final form: 30 January 2005)

Key words: binding delocalization, binding isotherm, continuous variation method, cyclodextrins, lattice model, poly(ethylene glycol), polymer inclusion complexes

Abstract

With polymer substrates, the continuous variation method based on monomer unit concentration underestimates the number of monomer units covered by a low-molecular-weight ligand. Accordingly, gel permeation chromatography confirms that an α-cyclodextrin (α-CD) molecule occupies more than previously claimed two ethylene glycol units in solid α-CD/poly(ethylene glycol) inclusion complexes. Consequently, the poly(ethylene glycol) chain cannot be modeled as an array of distinct binding sites corresponding to two monomer units and no preferred positions, i.e., no distinct binding sites probably exist on the chain for α-CD threading. The effect of such binding delocalization can be assessed using the theory of binding large ligands to a finite one-dimensional lattice [I.R. Epstein: *Biophys. Chem.* **8**, 327 (1978)]. Binding delocalization slightly decreases the average occupancy with highly occupied chains but strongly promotes the occupancy in the case of weak binding. This may be an additional reason for observed high yields of precipitated CD/polymer complexes.

Introduction

Cyclic oligosaccharides, cyclodextrins (CDs) are well known for their tendency to form inclusion complexes with various compounds [1]. Inducing precipitation of the complex from saturated α-CD solution by addition of poly(ethylene glycol) [PEG], Harada *et al.* [2, 3] demonstrated that compounds forming inclusion complexes with CDs include also linear synthetic polymers. Later on, complexes of other CDs with various other polymers were prepared, either in a similar way or using more sophisticated techniques [4]. Such complexes are denoted as pseudopolyrotaxanes because several CDs are threaded on a single polymer chain. Precipitation of pseudopolyrotaxanes is so prominent that the investigation of solid complexes still dominates the field although some attention has also been paid to formation of pseudopolyrotaxanes in solution.

A mechanism is conceivable in which threading of CDs proceeds, while the polymer chain is being built into precipitate; alternatively, it may be assumed that the chain is covered by CD before being incorporated into precipitate and no additional threading occurs on chains that entered the precipitate. A stricter requirement would be that all chains that ultimately form a precipitate are fully covered even before the precipitation starts [5, 6]. The differences between these possibilities are relevant only for kinetics of the solid precipitate formation; at overall equilibrium, fully covered chains in solution are in equilibrium with precipitate and, of course, equilibrium between all species in solution (free polymer, free CD and pseudopolyrotaxanes) is also established. In order to describe such equilibrium in solution, an adequate model of pseudopolyrotaxane formation has to be available that leads to a binding isotherm.

So far, several approaches to modeling pseudopolyrotaxane formation appeared in the literature. Molecular dynamics studies [7, 8] have revealed the effect of complexation on conformation of both CDs and included polymer chains and provide estimates of binding energy but do not lead to a binding isotherm. The Flory–Huggins lattice model has been applied to inclusion of polymer chains into prefabricated 'nanotubes' rather than to the formation of such 'nanotubes' from CD molecules in the presence of suitable polymer [9].

The last group of models of pseudopolyrotaxane formation deals with the kinetics of threading onto a linear chain of binding sites communicating with the surrounding solution only through terminal binding sites. The process is described as one-dimensional Fickian diffusion, the relevant set of equations being solved either numerically [10], or as a random hopping of ligands between binding sites simulated by the Monte Carlo method [11]. Obviously, the equilibrium properties could be retrieved for very long times. In initial applications of the models, binding isotherms were known beforehand because the models were used

* Author for correspondence. E-mail: horsky@imc.cas.cz

describing CD threading onto polymer chains that were actually divided into distinct binding sites by ionogenic groups functioning as passable dividers and the cooperativity need not to be taken into account.

We used the hopping model [12] in a general analysis of threading of asymmetric rings onto linear chains even though the diffusion model may seem more appropriate for polymers lacking distinct dividers. However, it is the hopping model into which the cooperativity of threading can easily be incorporated and the cooperativity of threading is considered to be a key reason for the observed high yields of precipitated CD/polymer complexes. One of the most important results of the study is that at equilibrium, a chain of binding sites communicating with a surrounding medium through terminal sites only is equivalent to a chain of freely accessible binding sites.

Although our theoretical study was general, we undertook it with α-CD and PEG in mind. We believed that modeling of PEG as a chain of distinct binding sites was justifiable on both theoretical and experimental grounds. While moving along a polymer chain, the threaded CD molecule experiences periodically changing interactions, which means that some positions of CD are preferred. This makes modeling of a polymer as a chain of binding sites more acceptable, and a correlation of spacing of these binding sites with the length of a monomer unit are to be expected. In this respect, literature gives credence to our choice because it is widely claimed that the stoichiometry of a solid α-CD/PEG complex is one CD molecule per two monomer units [13].

The stoichiometry was, however, inferred from a continuous variation plot and this method was demonstrated recently [14] to provide spurious results for cooperative or multistep binding. Threading of unmodified CDs onto polymer chains is assumed to be both a cooperative and multistep process. As the stoichiometry is essential for applicability of a hopping model to the complex, we determined the composition of solid α-CD/PEG complex prepared under various conditions using GPC separation. Although we obtained a higher PEG/α-CD ratio we were able to reconcile our results with the published results of the continuous variation method. Finally, we analyzed how the theoretical description of the threading process is affected by the fact that an α-CD molecule occupies a non-integral number of ethylene glycol monomeric units. We used the theory of binding large ligands to a finite one-dimensional lattice introduced by Epstein [15] and followed how binding delocalization affect the binding isotherm.

Experimental part

Materials

PEGs were of commercial origin (Fluka, Serva) with nominal molecular weights 600, 1000, 1500, 2000 which agreed with molecular weights determined by MALDI-TOF mass spectrometry with the exception of the nominal molecular weight 1000 for which about 10% higher value was obtained.

α-CD was purchased from Aldrich and was used as received; however, a sample was dried at 40 °C under reduced pressure and the found water content was taken into account in preparations of α-CD solutions.

Methods

Preparation

Stock solutions of all PEGs and α-CD were prepared with purified water (Millipore), mixed in appropriate ratios, and diluted with water if necessary. After mixing, the samples were shortly sonicated, left overnight at room temperature. Samples with precipitate were centrifuged and the supernatant was transferred to another vessel; inspection after two weeks detected no additional precipitation in any supernatant. In order to determine the amount of occluded supernatant, the precipitate was weighed both immediately after removal of the supernatant and after drying at 40 °C under reduced pressure.

Analysis

The composition of both precipitate and supernatant was determined by size exclusion chromatography (SEC) with water as a solvent. Since the complexation between PEG and α-CD is reversible, the solid complex (precipitate) can be redissolved in excess of water [3]. The equipment consisted of a VCR 40 KPLC pump (Academy Development Works, Prague, Czech Republic), a Model 7125 injection valve (Rheodyne, Cotati, CA), a R-401 differential refractometer (Waters, Milford, MA) and a stainless-steel column with hydrophilized GMB 100 poly(glycidyl methacrylate) packing (Labio Ltd. Prague, Czech Republic). Under the conditions used, the PEG peaks were well separated from that of α-CD (see Figure 1). Because the binding between α-CD and PEG is weak in dilute solutions [16], the initial concentration of the α-CD/PEG complex is negligible in all injected solutions containing both PEG and α-CD and the complex is ultimately decomposed in the course of GPC separation [17]. Thus, the concentrations of PEG and α-CD in precipitate and supernatant could be determined from the area under the peak after calibration with component solutions of known concentrations. The precipitate composition was corrected for retained supernatant.

Results and discussion

Yield and composition of pseudopolyrotaxanes

The yield of the solid inclusion complex of α-CD and PEG (pseudopolyrotaxane) at various α-CD concentrations and various PEG molecular weights is depicted in Figure 2. The data for PEGs of various molecular

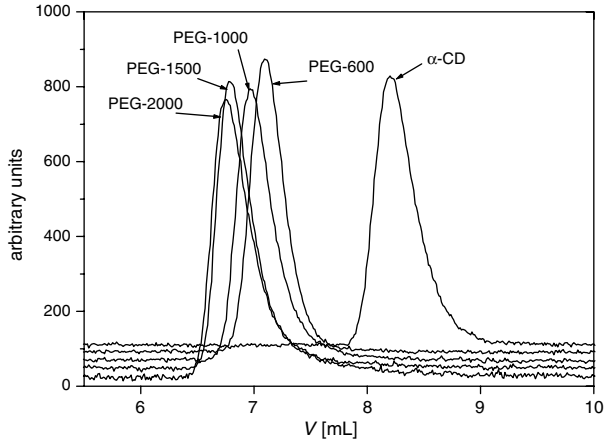

Figure 1. Overlaid SEC traces of α-CD and PEG samples, showing good separation of α-CD and PEG peaks.

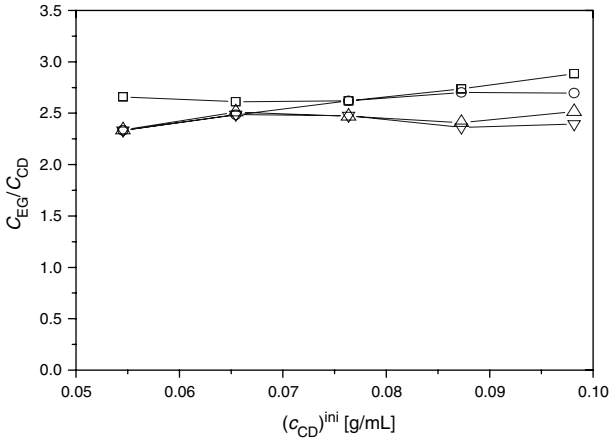

Figure 3. Average number of PEG monomeric units per CD in precipitated pseudopolyrotaxanes. For symbols, see Figure 2.

weights are almost identical; such an agreement was achieved only after the scatter of data was decreased by correcting the yield for retained supernatant. We also took great care to ensure that the precipitation of pseudopolyrotaxanes is carried to completion.

The composition of both the precipitated pseudopolyrotaxane and supernatant were determined by SEC. The information on supernatant was used for refining the data on the precipitate composition, summarized in Figure 3. The number of monomeric units per CD ring is higher than 2 in all cases, the average value being 2.53.

It can be argued that this finding does not necessarily mean that the ligand size is not equal to two monomer units because certain portions of PEG chains, in particular the terminal parts, may remained uncovered. The effect of bare ends should, however, decrease with molecular weight, as the contribution of the ends becomes less important. No such effect of molecular weight was observed. In analogous way, the number of random vacancies may be expected to decrease with increasing ligand concentration. Again, no decrease in the number of monomeric units per CD molecule was observed. Therefore, it is prudent to consider that one α-CD molecule covers more than two PEG monomeric units, especially because we are going to show in the following section that the continuous variation method as applied to polymer complexes underestimates the number of monomeric units covered by a ligand.

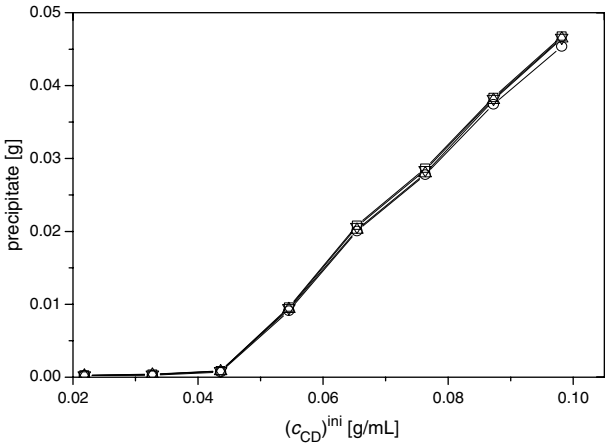

Figure 2. The amount of dried precipitate obtained from 1 ml of the solution initially containing 10 mg of PEG and α-CD at concentrations given as abscissa . Molecular weights of PEG: 600,◯;1000, Δ; 1500, ▽; 2000, □.

Application of continuous variation method to polymer complexes

The continuous variation method was widely used for determining the stoichiometry of inclusion complexes of CDs and various polymers. In its standard form, a physical quantity proportional to the product concentration is measured for a series of solutions in which the total molar concentration of reactants is kept constant, while their mole ratio is varied. The product composition is determined from the ratio at which the measured quantity attains the maximum. It can be shown that the amount of precipitated product can be used as a suitable measured quantity. In principle, there is no restriction on the product stoichiometry, and the continuous variation method can be used to determine the number of ligands bound to a polymer molecule. However, as the number of bound ligands could be rather high the maximum is located at the margin of the compositional span, and therefore difficult to determine. This may be the reason why with CD/polymer inclusion complexes, the continuous variation method based on the monomeric (repeating) units concentration instead of the molar polymer concentration has been used.

Such modification, while shifting the maximum towards the middle of the compositional span, tacitly assumes that the binding of ligands to polymer molecules can be described as binding of free monomeric units to a ligand. It has to be shown whether such assumption is adequate or what approximation it brings into the continuous variation method.

Let us assume non-cooperative one-step binding of n ligands to a polymer molecule composed of m mono-

meric units, which can be described by overall binding constant K

$$K = \frac{[C]}{[P][L]^n}, \quad (1)$$

where [P], [L], and [C] are molar concentrations of free polymer, ligand and complex, respectively. Setting $K' = K/m$ and [M] = m[P], we get

$$K' = \frac{[C]}{[M][L]^n}. \quad (2)$$

Assuming that R mols of precipitate is formed from a unit volume, we can write down the mass balances for the total concentration of a ligand, c_L, and of the polymer expressed as the concentration of monomer units, c_M,

$$c_L = [L] + n[C] + nR, \quad (3)$$

$$c_M = [M] + m[C] + mR. \quad (4)$$

The sum of polymer and ligand concentrations, c_{tot}, is kept constant and the overall composition is expressed by 'molar fraction' $x = c_L/c_{tot}$. Generalizing the procedure used by Huang *et al.* [14] for the case $m = n$, we arrive at the relation for the position of maximum precipitate x_{max}

$$\frac{x_{max}}{1 - x_{max}} = \frac{nc_{tot} - n(m-1)[C] - n(m-1)R}{c_{tot} + n(m-1)[C] + n(m-1)R}. \quad (5)$$

For large c_T, $R >> [C]$ and $R -> c_{tot}/(m+n)$ and Equation (5) becomes

$$\frac{x_{max}}{1 - x_{max}} = \frac{n+1}{m+1} = \frac{\frac{m}{s}+1}{m+1}, \quad (6)$$

where s corresponds to the number of monomer units covered by a ligand. Equation (6) shows that the continuous variation method based on monomer unit concentration underestimates the number of monomer units covered by a ligand. Thus, for $m = 11$, $x_{max} = 0.333$ corresponding to $s = 2.2$ rather than 2, the value obtained by standard interpretation of the continuous variation plot. Equation (6) does not fully explain the difference between the results of GPC and of the continuous variation method but shows that the latter results also confirm that one α-CD molecule covers more than two PEG monomeric units.

Binding of large ligands to a finite one-dimensional lattice

The above analysis of solid pseudopolyrotaxanes formed by PEG and α-CD indicates that there are no preferred locations of CD along a PEG chain, which, consequently, should be treated as a smooth wire when deriving the binding isotherm; naturally, a one-dimensional Fickian diffusion model comes to mind. Such a model would be kinetic in nature but equilibrium properties could be retrieved at infinite time. The model would also require disregarding cooperative interactions but this is acceptable for the initial analysis of the effect of binding delocalization. The limiting factor, however, is that in this case the diffusional distance is comparable with the dimension of diffusant because, e.g., PEG 1000 can accommodate only about 10 CD molecules.

Therefore, we use a model for cooperative binding of large ligands to a finite one-dimensional lattice proposed by Epstein [15]; large is to be understood as binding of one ligand to a multiplet of binding sites. McGhee and von Hippel [18] showed already 30 years ago that such a multiplet cannot be simply aggregated in 'binding supersite' because in doing so we would disregard many configurations in which ligands span across 'supersite' boundaries. Epstein carried out a combinatorial analysis of cooperative large-ligand binding to a finite one-dimensional lattice, which gives an expression for the probability that there are kN-site ligands on a M-site lattice with j adjacencies

$$p(M,N,k,j) = \frac{P(M,N,k,j)(KL)^k\omega^j}{1 + \sum_{l=1}^{g}\sum_{i=s}^{l-1} P(M,N,l,i)(KL)^l\omega^i}, \quad (7)$$

where K is a equilibrium constant for binding of a ligand to an isolated N-plet, L is the free ligand concentration, ω is a cooperativity parameter, and P(M,N,k,j), the number of distinct ways that k N-site ligands may bind to an M-site lattice with j adjacencies, is given as

$$P(M,N,k,j) = \frac{(M - Nk + 1)!(k-1)!}{(M - Nk - k + j + 1)!(k-j)!j!(k-j-1)!}. \quad (8)$$

The index of the outer summation in the denominator of the right-hand side of Equation (7) is running to g which is the greatest integer less than or equal to M/N. The starting value, s, of the inner summation, was given by Epstein as 0 but, of course, adjacencies cannot be avoided with almost saturated lattices because there are simply not enough vacant binding sites in some cases, and therefore s should read

$$s = 0 \quad \text{for } M - Nl > l - 2$$

$$l(N+1) - M - 1 \quad \text{for } M - Nl < l - 1. \quad (9)$$

The probability that there are exactly k ligands bound to the lattice, regardless of the number of the ligand adjacencies, is simply

$$p(M,N,k) = \sum_j p(M,N,k,j) \quad (10)$$

and the average number of ligands per lattice is

$$\bar{k} = \sum_k kp(M,N,k) \quad (11)$$

We are going to use the above relationships to find the effect of binding delocalization on the binding isotherm. Delocalization will be simulated in the scope of the Epstein model by increasing the number of binding sites covered by a ligand, while preserving all other relevant parameters – the binding constant K, the cooperative parameter ω and, most importantly, the relative length of a lattice in terms of the ligand size M/n. The results for a chain with the length corresponding to 10 ligands are presented in Figure 4. The decrease in the size of an elementary step by which the ligand can move along the chain has the effect which depends on the occupancy of the chain. At low occupancy, the effect is strongly promoting, whereas for almost fully covered chains the average occupancy is decreased. The latter is understandable because if the distance between two neighboring ligands is less than the length of the ligand that portion of the chain is inaccessible to any other ligand. The probability of such small gaps increases with the lattice occupation.

Such behavior applies both to uncooperative and cooperative binding even though the cooperativity goes against delocalization by restricting free movements of ligands. Application of the model to cooperative binding of a ligand corresponding to many binding sites is somewhat questionable because the model assumes that the cooperative interaction is switched off as soon as the ligands are separated even by a single elementary step which represents a few picometers for CD molecule corresponding to hundreds binding sites. The qualitative picture of the delocalization effect given above, however, is not affected and the binding isotherms for large ligands are more gradual (Figures 5 and 6) than those for fully localized binding ($N = 1$).

The suggested use of polymer/CD inclusion complexation as a force generator in a nanoscale machinery [19] assumes that the hydrogen bonding between adjacent threaded CD rings is the source of cooperativity and, consequently, the reason for high solid complex yields; however, an alternative explanation of high yields was suggested, namely, low solubility of the complex. The above analysis based on the Epstein model shows that there could be yet another reason for high yields – binding delocalization due to which the average occupancy can be strongly increased even if the binding of a single ligand is very weak. On the other hand, we have shown that delocalization of binding has an anticooperative effect at high occupancy and the chains fully covered by CD are those that are supposed to precipitate. Thus, what matters is the concentration of fully covered chains, i.e., we have to deal not only with the average degree of binding but also with the degree of binding distribution. In Figure 7 two distributions, giving the identical value of an increased average bind-

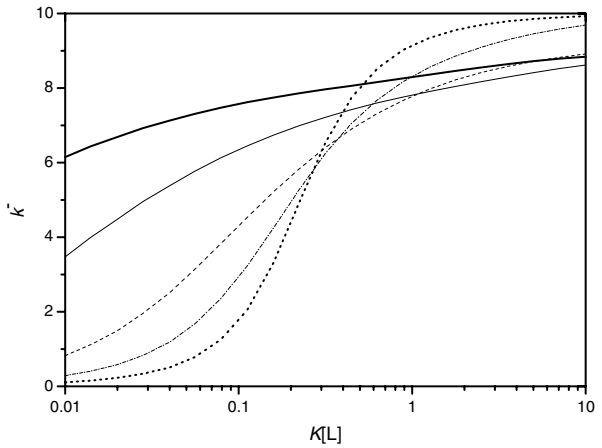

Figure 5. The effect of delocalization on the average number of ligands per chain $\bar{k}$ for cooperative formation of pseudopolyrotaxanes. The lattice has the length of 10 ligands; the cooperativity parameter $\omega = 5$. The ligand covers 1 (dotted line), 3 (dot dashed line), 10 (dashed line), 100 (thin line), or 1000 (thick line) elementary binding sites.

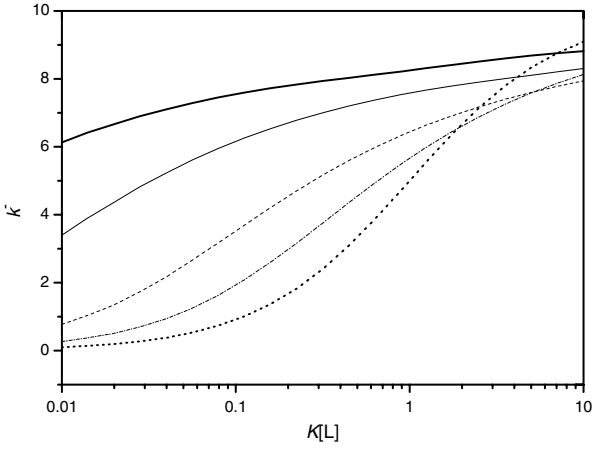

Figure 4. The effect of delocalization on the average number of ligands per chain $\bar{k}$ for non-cooperative formation of pseudopolyrotaxanes. The lattice has the length of ten ligands. The ligand covers 1 (dotted line), 3 (dot dashed line), 10 (dashed line), 100 (thin line), or 1000 (thick line) elementary binding sites.

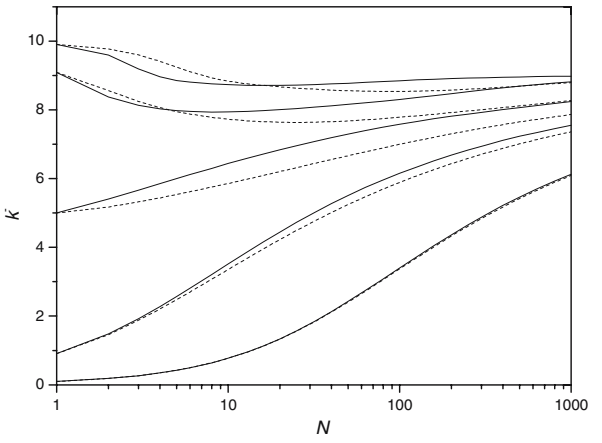

Figure 6. The effect of binding delocalization on the average number of ligands per chain $\bar{k}$ for cooperative formation of pseudopolyrotaxanes. The lattice has the length of 10 ligands; the cooperativity parameter $\omega = 1$ (full lines) or $\omega = 5$ (dashed lines). For $\omega = 1$, the values of K[L] are 0.01, 0.1, 1, 10, 100 (going from bottom up). Values for $\omega = 5$ were chosen to provide the same average number of ligands per chain for ligands with the size $N = 1$.

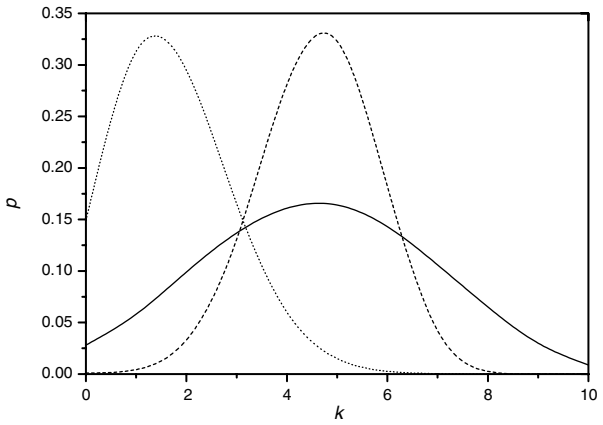

Figure 7. The effect of binding delocalization on the occupancy distribution, expressed as a fraction p of chains having k threaded rings. The chain length corresponds to 10 ligands and $N = 1$, $\omega = 1$ for dotted line (non-cooperative localized binding), $N = 10$, $\omega = 1$ for dashed line (non-cooperative delocalized binding) and $N = 1$, $\omega = 5$ for full line (cooperative localized binding). The value of K[L] was set to 0.210 at which the average number of threaded rings $\bar{k} = 4.61$ both for non-cooperative delocalized and cooperative localized binding.

ing, are compared and it is seen that the distribution is much narrower if the increase is achieved by binding delocalization rather than by cooperativity. In fact, the fraction of fully covered chains is lower for the delocalized binding than for fully localized binding. Thus, although delocalization of binding strongly increases an average degree of binding, it does not promote formation of fully covered chains, and therefore does not promote their precipitation.

Conclusion

Even though we presented a strong case for α-CD covering non-integer number of monomeric units in α-CD/PEG complex, the matter is not settled because it is not known whether the precipitate of this complex is composed of fully covered chains only. Nevertheless, the binding delocalization must be taken into account in analysis of binding isotherms of pseudopolyrotaxanes, not only for those of α-CD and PEG but, in general, because non-integer stoichiometry was already reported for α-CD inclusion complexes with some other polymers. [11]

Acknowledgement

The project was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (Grant A1050101).

References

1. J. Szejtli: *Chem. Rev*. **98**, 1743 (1998).
2. A. Harada, and M. Kamachi: *Macromolecules* **23**, 2821 (1990).
3. A. Harada, J. Li, and M. Kamachi: *Macromolecules* **26**, 5698 (1993).
4. A. Harada, M. Okada, Y. Kawaguchi, and M. Kamachi: *Polym. Adv. Technol*. **10**, 3 (1999).
5. M. Ceccato, P. Lo Nostro, and P. Baglioni: *Langmuir* **13**, 2436 (1997).
6. P. Lo Nostro, J.R. Lopes, and C. Cardelli: *Langmuir* **17**, 4610 (2001).
7. J. Pozuelo, F. Mendicuti, and W.L. Mattice: *Macromolecules* **30**, 3685 (1997).
8. J. Pozuelo, F. Mendicuti, and W.L. Mattice: *Polym. J*. **30**, 479 (1998).
9. Y. Okumura, K. Ito, and R. Hayakawa: *Phys. Rev. Lett*. **80**, 5003 (1998).
10. L.P. Meier, M. Heule, W.R. Caseri, R.A. Shelden, U.W. Suter G. Wenz, and B. Keller: *Macromolecules* **29**, 718 (1996).
11. W. Herrmann, B. Keller, and G. Wenz: *Macromolecules* **30**, 4966 (1997).
12. J. Horský: *Macromol. Theory Simul*. **9**, 759 (2000).
13. A. Harada: *Carbohydr. Polym*. **34**, 183 (1997).
14. C.Y. Huang, R.X. Zhou, D.C.H. Yang, and P.B. Chock: *Biophys. Chem*. **100**, 143 (2003).
15. I.R. Epstein: *Biophys. Chem*. **8**, 327 (1978).
16. J. Horský: *Eur. Polym J*. **34**, 591 (1998).
17. X. Lou, Q. Zhu, Z. Lei, J.L.J. Dongenvan, and E.W. Meijer: *J. Chromatogr. A*. **1029**, 67 (2004).
18. J.D. McGhee, and P.H. Hippelvon: *J. Mol. Biol*. **86**, 469 (1974).
19. H. Fujita, T. Ooya, M. Kurisawa, H. Mori, M. Terano N. Yui: *Macromol. Rapid Commun*. **17**, 509 (1996).