Synthesis of polyrotaxanes based on α-cyclodextrin and poly(ethylene oxide)

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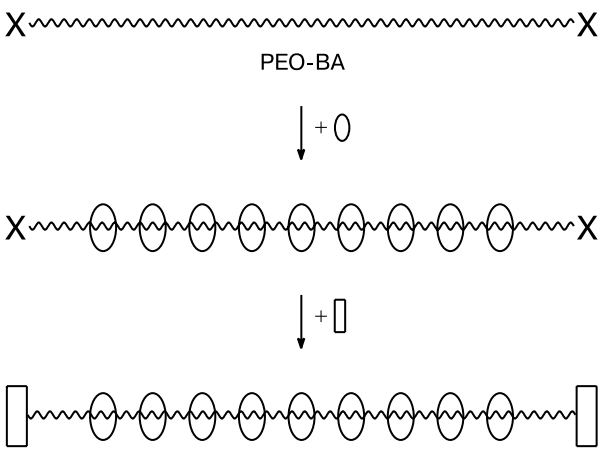
Inclusion complexes of poly(ethylene oxide) with α-cyclodextrin are the key compounds in the synthesis of polyrotaxanes. These complexes prepared in aqueous solutions contain free cyclodextrin, which cocrystallizes with the major reaction product. These complexes dissociate upon dissolution in DMF and DMSO to form cyclodextrin and pseudopolyrotaxanes with a low cyclodextrin content. Polyrotaxane was synthesized with the use of poly(ethylene oxide)-α,ω-bis-amine as a linear component. The end-groups of the polymer in the inclusion complex were modified by the reaction with 2,4-dinitrofluorobenzene. A procedure was developed for purification of a polyrotaxane with high cyclodextrin content.

Key words: supramolecular structures, supramolecules, inclusion complexes, polyrotaxanes, cyclodextrins.

Molecular self-assembly is one of the main principles of formation of complex biological structures. In chemistry, this principle is successfully applied to the design of new molecular assemblies and nanometer-sized particles. In the resulting supramolecular structures, the constituent molecules are linked to each other by noncovalent bonds.[1,2] This class of compounds includes rotaxanes, which are supramolecular compounds containing cyclic components (rotors) threaded onto an axle molecule (rod). Bulky substituents (stoppers) are added at the ends of the axle molecule to prevent cyclic molecules from sliding off.[3] Unlike pseudorotaxanes, which are topologically similar to rotaxanes but contain no stoppers, rotaxanes can be isolated from reaction mixtures avoiding the undesired step of dissociation into the starting components. Polyrotaxanes (PR) are in principle constructed analogously to rotaxanes but contain a larger number of cyclic molecules threaded onto a rod (generally, onto a polymer chain). The use of PR as important elements of electroconducting, luminescent, and other nanostructures was covered in the review.[4]

Polyrotaxanes formed by threading cyclic molecules, *viz.*, cyclodextrins (CD), onto poly(ethylene oxide) (PEO)[5] or poly(ethylene oxide)—poly(propylene oxide) block copolymers[6] have been documented. Polyrotaxanes based on cucurbiturils were synthesized by linking cucurbituril complexes with low-molecular-weight guests.[7,8] These reactions also afford closed supramolecular structures, *viz.*, catenanes,[7] as by-products. In the present study, we investigated the synthesis of PR by threading CD molecules onto PEO (Scheme 1).

Scheme 1



PEO-BA is poly(ethylene oxide)-α,ω-bis-amine, ○ is α-cyclodextrin, ▯ is a stopper

Polyrotaxanes were synthesized according to Scheme 1 with the use of α-CD and poly(ethylene oxide)-α,ω-bis-amine (PEO-BA) in two steps. In the first step, an inclusion complex based on PEO-BA and α-CD was prepared.

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Then stoppers were attached to the amino groups of the polymer. This is the key step in the synthesis of PR, because it can be accompanied by sliding off of α-CD molecules from the polymer chain. The aim of the present study was to search for conditions for the introduction of stoppers into pseudopolyrotaxanes (PPR) characterized by minimum sliding off. This was achieved by changing the phase state of the reaction system in the course of the chemical modification of the end-groups of the polymer involved in PPR.

Experimental

α-Cyclodextrin (α-CD, Sigma) dried *in vacuo* and poly(ethylene oxide)-α,ω-bis-amine (PEO-BA, Sigma, synthesized from poly(ethylene oxide) with $M_w = 3350$) were used. The molecular weight of PEO-BA was refined by MALDI-TOF mass spectrometry on a VISION 2000* instrument (TermBioAnalysis), and the correspondence between the amounts of the amino groups in the polymer and the theoretical value was estimated by titration with hydrochloric acid. The results of analysis are presented in Figs 1 and 2. The end-groups were modified with 2,4-dinitrofluorobenzene (DNFB, Serva) used without additional purification. All solvents were purified by distillation; DMF and DMSO were distilled *in vacuo*. The model compound, *viz.*, 2,4-dinitro-*N*-ethylaniline, which was used for the determination of the extinction coefficient of the end-groups in PR, was synthesized by triturating DNFB with ethylamine according to a procedure described earlier.[9]

The complexes were prepared by mixing concentrated aqueous solutions of PEO-BA and α-CD. The precipitate was separated by centrifugation and washed with cold water to remove unconsumed reagents. The complexes were dried *in vacuo* over calcium chloride. Stoppers were attached according to three methods.

1. Synthesis in solution. A solution of a complex of α-CD with PEO-BA (46 mg) in DMF (1.5 mL) was mixed with a solution of DNFB (~20-fold excess, 9 mg) in DMF (0.5 mL). The mixture was kept at room temperature for 1 h and then poured into diethyl ether. The precipitate that formed was washed with diethyl ether and then washed repeatedly with acetone to separate unconsumed DNFB. The washed precipitate was dried *in vacuo*. The yield was 76%, the product is soluble in water and DMF.

2. Synthesis in suspension. The solid α-CD—PEO-BA complex (380 mg) was added to a solution of DNFB (88 mg, 25-fold excess) in DMF (5 mL) and the reaction mixture was stirred at room temperature for 1 day, PPR remaining partially undissolved. The reaction mixture together with the precipitate was poured into diethyl ether and washed as described above. The yield of the solid product was 87%. If the insoluble precipitate was separated and washed, the yield of the product was 19% with respect to the weight of the starting complex. After thorough purification, the yield of PR was 10—15%.

3. Solid-phase synthesis. The reaction was carried out by triturating a mixture of the dry complex of α-CD with PEO-BA (250 mg) and DNFB (250 mg) in a mortar, after which the reaction mixture turned yellow. After 10 min, the resulting PR was washed from an excess of DNFB with acetone and dried *in vacuo*. The yield of the crude product was 85%. To purify from α-CD, the precipitate was washed with DMF until the washings became almost colorless. The yield of PR was 10% with respect to the weight of the starting complex.

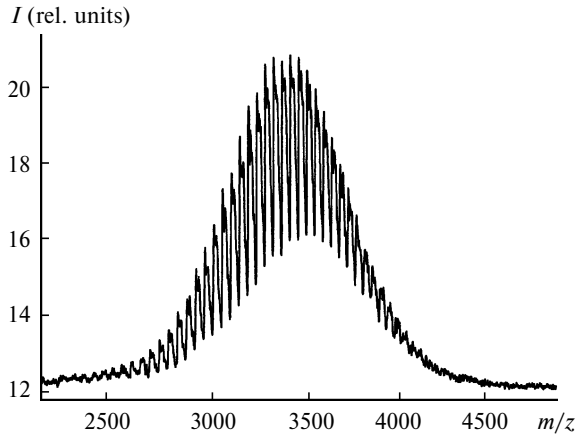


Fig. 1. Mass spectrum of PEO-BA.

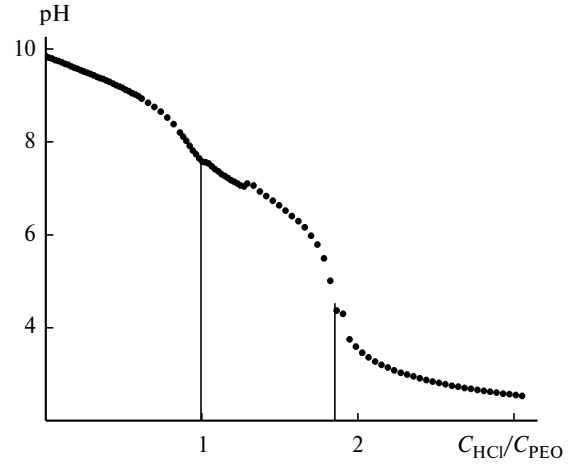


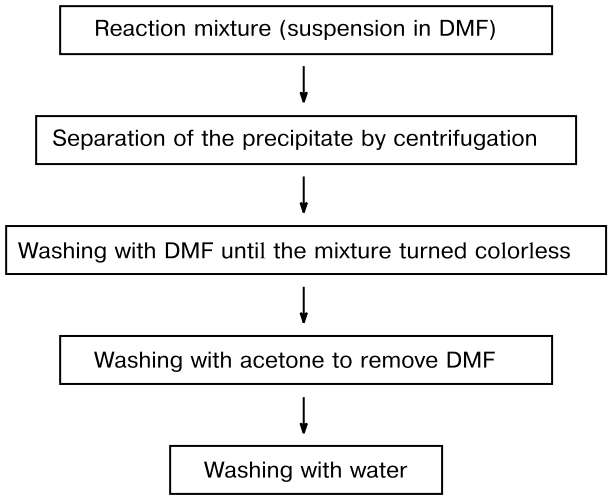
Fig. 2. Curve of acid-base titration of PEO-BA with hydrochloric acid.

Determination of the composition of polyrotaxane. The α-CD : PEO ratio in polyrotaxanes was determined by ^{1}H NMR spectroscopy on a Bruker DRX-500 spectrometer (500 MHz) in DMSO-d_6 based on the ratio of the area of several signals corresponding to the protons of cyclodextrin to the area of the signal of the methylene protons of PEO. The assignment of the signals for the protons of α-CD was made using the reported data.[6] The concentration of the dinitrophenyl end-groups in PR was determined spectrophotometrically (λ_{max} = 360 nm) in DMF. In some cases, the concentration of the end-groups was evaluated by ^{1}H NMR spectroscopy.

Polyrotaxanes were isolated according to Scheme 2, which allows one to completely remove low-molecular-weight impurities.

* The measurements were carried out at the M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences.

Scheme 2



Results and Discussion

The formation of PPR from α-CD and PEO, which is the first step in the synthesis of polyrotaxane, has been described in detail.[10] Mixing of aqueous PEO-BA and α-CD solutions also afforded a crystalline precipitate of the complex in high yield.[11] In the present study, most attention was given to the chemical modification of the end-groups in PPR. Generally, the reactions are performed[5,6,12] in DMF or DMSO. Since these solvents are unfavorable for hydrophobic interactions between guest and host molecules, which are among the driving forces for complexation, the modification reaction is accompanied by sliding the α-CD molecules off the polymer chain during dissolution of PPR. Actually, it has earlier been demonstrated[13] that the attachment of stoppers was accompanied by sliding off about 25% of α-CD molecules. In this connection, we attempted to find conditions for the modification under which sliding off will be reduced to minimum.

Recently, it has been found that the nucleophilic substitution readily occurs in the solid phase. For example, oxazolidines, perimidines, and Schiff bases were synthesized according to this method in high yields.[9] In addition, trituration of a dry mixture of components is widely used for the synthesis of inclusion complexes of low-molecular-weight compounds with α-CD.[14] Hence, we modified the terminal groups in PPR by the solid-phase method (method 3) and compared the characteristics of the polymer thus synthesized with those obtained in solution (method 1) and suspension (method 2).

The compositions of the samples were determined by ^{1}H NMR spectroscopy and spectrophotometry. The amount of α-CD was determined from the intensities of the signals at δ 3.6—5.8. The percentage of PEO was evaluated from the intensity of the signal for the methylene protons (δ 3.5). In the case of PR with low α-CD content, the amount of dinitrophenyl groups was also estimated from the ^{1}H NMR spectra based on the signals for the aromatic protons at δ 7—9. In other cases, this parameter was determined spectrophotometrically.

The properties of samples of PR prepared by methods 1—3 differ. For example, the solubilities of PR synthesized in the solid phase and suspensions are much lower than those of the samples prepared in solution. This is also evidenced by the data[5] according to which purified PR is insoluble in DMF. The samples synthesized in solution, unlike the other samples, are well soluble also in water. All this suggests that the number of threaded α-CD molecules in PR depends on the procedure for the synthesis of the latter. The higher the content of α-CD in rotaxane, the lower solubility of PR in DMF and water.

The quality of the ^{1}H NMR spectra of PR synthesized according to method 1 is substantially better than that of the samples prepared according to the other two methods: distinct splitting of the signals for the aromatic protons and protons of α-CD was observed, whereas signals in the spectra of the samples synthesized by the other two methods are noticeably broadened. The spectrum of the sample prepared according to method 1 is identical to the spectrum of the starting PPR (except for the signals for the additional aromatic protons). This is evidence that the interaction between the α-CD molecules in this sample is virtually identical to that in the starting complexes. In DMSO used for measuring spectra, the complexes dissociate into the starting components. Hence, it can be stated that it is α-CD molecules, which are either not involved in PPR or are treaded onto the polymer chain and move freely along this chain, that are predominantly recorded in the spectra. Broadening of the signals in the ^{1}H NMR spectra of the other samples may be attributed to the fact that, in the case of high α-CD content, hydrogen bonds are formed between the adjacent molecules, which limits mobility of protons. The broadening can also be attributed to low solubility of PR in DMSO, resulting in the formation of intermolecular associates. Apparently, both these effects take place in PR with high α-CD content.

It is practically impossible to completely purify the starting complex because it is noticeably soluble in water, dissolution being accompanied by its partial dissociation. As a result, a considerable amount of the complex goes into solution after several washings. Nevertheless, the determination of the α-CD content in the complex after washing with water allows one to draw a conclusion about the composition of PPR. Actually, if only the complex goes into solution upon treatment with water, this should not lead to a change in the ratio of the components in PPR that remains insoluble. However, analysis of the ^{1}H NMR spectra shows that the number of α-CD molecules in the complex decreases already after the first washing. For example, there are 2.54 ethylene oxide units

per α-CD molecule in the starting complex, whereas the number of this units in a sample obtained after single washing is 2.63. In the washings, this ratio is 1.44, *i.e.*, α-CD is predominantly washed out during purification. This suggests that precipitation of PPR is accompanied by cocrystallization of the complexes with unconsumed α-CD. Similar suggestions have been made earlier.[15]

The PEO unit : α-CD ratio for different polyrotaxane samples were determined from the ^{1}H NMR spectra. This ratio is approximately the same for the samples synthesized by different methods and for the starting PPR (2.5—2.7 PEO units per α-CD). It should be noted that the PEO unit : α-CD ratio estimated by calculations[16] and experimentally[5] varies from 2.3 to 2.5. From the data on the molecular modeling it follows that two PEO units could fit into a α-CD molecule. Experimentally, this ratio was obtained only for complexes based on PEO with a molecular weight of 600—1500.[5] Since all samples, which were not subjected to thorough purification, have nearly the same empirical composition, the difference in their properties can be attributed to the presence of free α-CD in the samples as well as with the difference in the ratio of the free to bound α-CD molecules.

Samples of PR prepared by the solid-phase and suspension syntheses were treated with water, which made it possible to remove both free α-CD and polyrotaxanes with low α-CD content. The ^{1}H NMR spectrum of the purified product prepared by the solid-phase synthesis differs from the spectrum of the starting reaction product and is identical to the spectrum of polyrotaxane purified by chromatography.[6] This is indicative of a rather high degree of purification of the samples isolated by washing with water.

The PEO unit : α-CD ratio for the sample prepared by the solid-phase synthesis followed by washing with water was 6 : 1. Since dissociation of PR into the starting components under the reaction conditions is completely excluded, this ratio can be attributed only to the fact that washing is accompanied by removal of a considerable amount of free α-CD molecules present in the starting PPR. This effect can be observed only in the solid-phase synthesis, because in the case of the synthesis in suspension, free α-CD is dissolved in DMF and is separated already during the synthesis.

We failed to purify the sample prepared in solution by washing with water and DMF because of its high solubility in these solvents. Taking into account high solubility and high mobility of protons in α-CD molecules, it can be concluded that the synthesis according to this method produces mixtures of free α-CD and polyrotaxanes with low content of α-CD. To analyze the differences between free and bound α-CD, we recorded the ^{1}H NMR spectra for a series of mixtures of PPR and α-CD in DMSO-d_6. An increase in the concentration of α-CD leads to virtually no changes in the positions of the peaks but results in better resolution of the signals for the protons of α-CD. This suggests that a solution of PPR in DMSO contains, along with free α-CD, a certain amount of α-CD molecules, which are threaded onto the polymer and easily move along the chain, *i.e.*, the complex dissociates only partially and a small amount of the complex with low α-CD content is present in solution. Apparently, it is this complex that gives PR in the synthesis in solution.

After thorough washing with DMF and water of the sample synthesized in suspension, a product insoluble in water, DMF, and DMSO was obtained. Nevertheless, chromatographic purification of PR in DMSO has been described.[5] Our experiments on purification of samples on Sephadex G-50 in DMSO demonstrated that dissolution of PR in this solvent gives rise to a slightly opalescent solution. A yellow fraction adsorbed at the top of the column was not eluted even after passing of the solvent over a long period of time. Hence, it follows that insoluble PR was adsorbed on the gel and was not eluted, whereas it was PR with low content of α-CD that was purified by chromatography.

To determine the composition of PR synthesized according to method 2, the ^{1}H NMR spectrum of the sample were recorded in a concentrated NaOD solution. Heating of PR in this solution causes elimination of the dinitrophenyl end-groups followed by liberation of α-CD and PEO-BA molecules. The resolution of this spectrum is substantially worse than that of the spectra in DMSO. For this reason and also because of low content of the end-groups, we failed to quantify them in the sample. However, the above-described properties of this PR suggest that there are no incompletely substituted end-groups in the sample. The PEO unit : α-CD ratio in this product was 3 : 1, which is lower than that found earlier[17] for analogous PR (3.9 : 1). Hence, the use of the suspension method allows one to prepare PR with high content of α-CD.

Successful modification of the end-groups in a PPR—DNFB suspension requires that the rate of dinitrophenylation was higher than the rate of sliding α-CD off the polymer chain. We studied the kinetics of dinitrophenylation. For this purpose, we mixed solutions of PPR and a 20-fold excess of DNFB in DMF. Then samples were withdrawn at intervals and poured into diethyl ether. The precipitate containing PR was washed with acetone, dried, and dissolved in DMF. The specific absorbance was measured at the wavelength corresponding to the absorption maximum of the dinitrophenyl group. The first sample was withdrawn within 2 min after mixing of the reagents; the maximum time of the contact was 40 h.

It appeared that the absorbance of the products remains virtually unchanged throughout the time range, *i.e.*, the modification of the end-groups occurs immediately after mixing of the reagents, and all groups acces-

sible for replacement are involved in the reaction. At the same time, the spectra of mixtures of PPR with an excess of α-CD showed that sliding α-CD off the polymer chain occurs incompletely to give a mixture of products, in which PPR with low content of α-CD molecules exist in equilibrium with free α-CD molecules. Analysis of the PR showed that in the case of the synthesis in solution, dissociation occurs in the step of dissolution of PPR before the addition of DNFB and sliding the major portion of α-CD molecules off the chain occurs before the attachment of the stoppers. In the case of the synthesis in suspension, dissociation has no time to occur and the reaction affords PR with high content of α-CD.

To conclude, we demonstrated that pseudopolyrotaxanes prepared by the reactions of α-CD with PEO in aqueous solutions involve unconsumed α-CD, which cocrystallizes with the major reaction product.

Study by ^{1}H NMR spectroscopy showed that dissolution of PPR in such solvents as DMF and DMSO is accompanied by their partial dissociation into the starting components to form complexes with low content of α-CD molecules, which slide freely along the chain.

Three methods for the synthesis of polyrotaxanes were compared. The synthesis in suspension giving rise to polyrotaxane with high α-CD content was demonstrated to be the method of choice.

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