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# Chiral Recognition of Poly(*N*-isopropylacrylamide-*co*-(D or L)-*N*-tryptophan−acrylamide) with Methylated β-Cyclodextrin

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Chiral recognition is an important subject in biological, medical, or pharmaceutical systems. Focusing on natural amino acids, their optical purity or ee values normally are determined analytically with chiral columns.[1−3] Cyclodextrins (CDs) are chiral, naturally occurring cyclic oligosaccharides, which, due to the unique molecular structure, are able to form inclusion (host−guest) complexes with various polymers.[4,5] The first stereoselective inclusion of a whole polymer chain with CDs was shown by Tonelli et al., who demonstrated that only isotactic poly(3-hydroxy butyrate) can be included in a α-CD cavity, while atactic poly(3-hydroxy butyrate) cannot.[6] Accordingly, the enantioselective enclosing of isotactic polylactides with CD rings was published by Yui et al. in 2007.[7] We demonstrated recently that in the case of a free radical polymerization of a CD complexed *N*-methacryloyl-D,L-phenylalanine methyl ester racemic mixture the D-enantiomer polymerizes preferentially, whereas the L-enantiomer tends to accumulate in the solution.[8]

Up to now, the enatioselective inclusion of chiral side groups of synthetic polymers with CD has not been investigated. Herein, we demonstrate the chiral recognition of the polymeric bound amino acid with RAMEB-CD (random methylated β-cyclodextrin). Generally, the glucose-based CDs might distinguish between native D- and L- amino acid enantiomers in aqueous media due to diastereomeric effects.[9−12] Thus, D- and L-tryptophan (D- and L-Trp) became the focus of our interest as chiral model compounds. It is an essential amino acid implemented in proteins with an aromatic moiety and therefore suitable to build inclusion complexes with RAMEB-CD.[13−15] Also, L-Trp is a precursor of serotonin, which is commonly used in pharmaceutical application as a tranquillizer. Furthermore, because of the presence of two functional domains, the amino and carboxylic groups D- and L-Trp are an interesting species for polymer synthesis.

The D- and L-Trp-based monomers are synthesized when the amino groups of the amino acid are modified via condensation reaction, while the free carboxylic group, sensitive to pH value, still remains. The remarkable polymer properties based on poly(*N*-isopropylacrylamide) (poly(NIPAAm)) induced us to select the NIPAAm as comonomer for direct copolymerization procedures. It is well-known that poly(NIPAAm) is a typical thermoresponsive polymer exhibiting a lower critical solution temperature (LCST) behavior in aqueous media at around 32 °C.[16] Above this critical temperature the polymer precipitates a stage that is reversible as soon as the temperature sinks below the LCST. The important feature is that the poly(NIPAAm) LCST value bears a tunable character due to the existence of a delicate hydrophilic/hydrophobic balance in its molecular structure. If the equilibrium is slightly altered, there is a possibility to vary that critical temperature.[17] We detected that the presence of D- or L-Trp in the copolymer largely affects this typical LCST value. Additionally, the presence of RAMEB-CD as ligand of side groups also influences the LCST, which is dependent on the stability of the complexes. Accordingly, we applied simple turbidity measurement to assign the polymer attached to D- or L-Trp enantiomers. Further, the dynamic light scattering (DLS) technique was used to measure particle size, which strongly depends on the degree of RAMEB-CD complexed side groups as well as UV spectroscopy to affirm the enantiomeric discrimination.

Thus, the synthetic pathway to the desired pH- and temperature-sensitive copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ containing chiral D- or L-Trp segments and correspondingly their supramolecular systems $\mathbf{4_D}$ and $\mathbf{4_L}$ with RAMEB-CD is represented in Scheme 1. At first, *N*-acryloyl-D-tryptophan ($\mathbf{2_D}$) and *N*-acryloyl-L-tryptophan ($\mathbf{2_L}$) were obtained under Schotten−Baumann conditions by using acryloyl chloride. Then, $\mathbf{2_D}$ or $\mathbf{2_L}$ was radically copolymerized with NIPAAm in different molar ratios (1:10, 1:15, and 1:20) under similar conditions. The structures of the synthesized compounds have been proven by spectroscopic methods, elemental analysis, and polarimetric measurements (Supporting Information). The molecular weights and molar weight distribution of polymers $\mathbf{3_D}$ and $\mathbf{3_L}$ were determined by means of gel permeation chromatography (GPC) showing similar results. The GPC measurements were carried out after silylation of the free carboxylic groups with trimethylchlorosilane (Supporting Information).

The formation of inclusion complexes with monomers either $\mathbf{2_D}$ or $\mathbf{2_L}$ Trp aromatic side groups have been confirmed by 2D ROESY spectroscopy. For this procedure we chose purified 2,6-dimethyl-β-cyclodextrin (DIMEB-CD) because of better water solubility compared to unmodified β-CD.

The 2D NMR measurement shows the correlation between protons of DIMEB-CD and the aromatic moiety of $\mathbf{2_D}$. Figure 1 indicates (as an example for $\mathbf{2_D}$) that no interaction of other functional groups of the monomer, like the double bond or the amino group with the DIMEB-CD cavity, occurs. Therefore, it can be concluded that the complex composition with $\mathbf{2_D}$ or $\mathbf{2_L}$ is of 1:1 stoichiometry. Unfortunately, NMR titrations for binding constants investigation could not be carried out since the complexation is only detectable in aqueous media. The Trp derivatives $\mathbf{2_D}$ and $\mathbf{2_L}$ are almost water-insoluble; therefore, a J-Plot as preferred method could not be used. We thus show the diastereomeric complex formation by UV measurements.

Figure 2 shows characteristic UV-absorption spectra for the copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ (1:20) aqueous solutions that were registered in the absence and presence of RAMEB-CD. One can see that UV absorptions are sensitive to the formation of different kinds of diastereomeric complexes. As expected, the uncomplexed copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ solutions exhibited identical absorptions. In contrast, the copolymers complexed with RAMEB-CD caused an increase of the UV absorption of the polymer-bound L-enantiomer $\mathbf{4_L}$ while the absorption of the D-enantiomer $\mathbf{4_D}$ decreased.

Further, the change of single coil dimensions and temperature controlled solubility (LCST) of the prepared polymeric compounds ($\mathbf{3_{D,L}}$) and correspondingly complexed with RAMEB-CD ($\mathbf{4_{D,L}}$) were investigated via DLS, thermal titration, and turbidity measurements.

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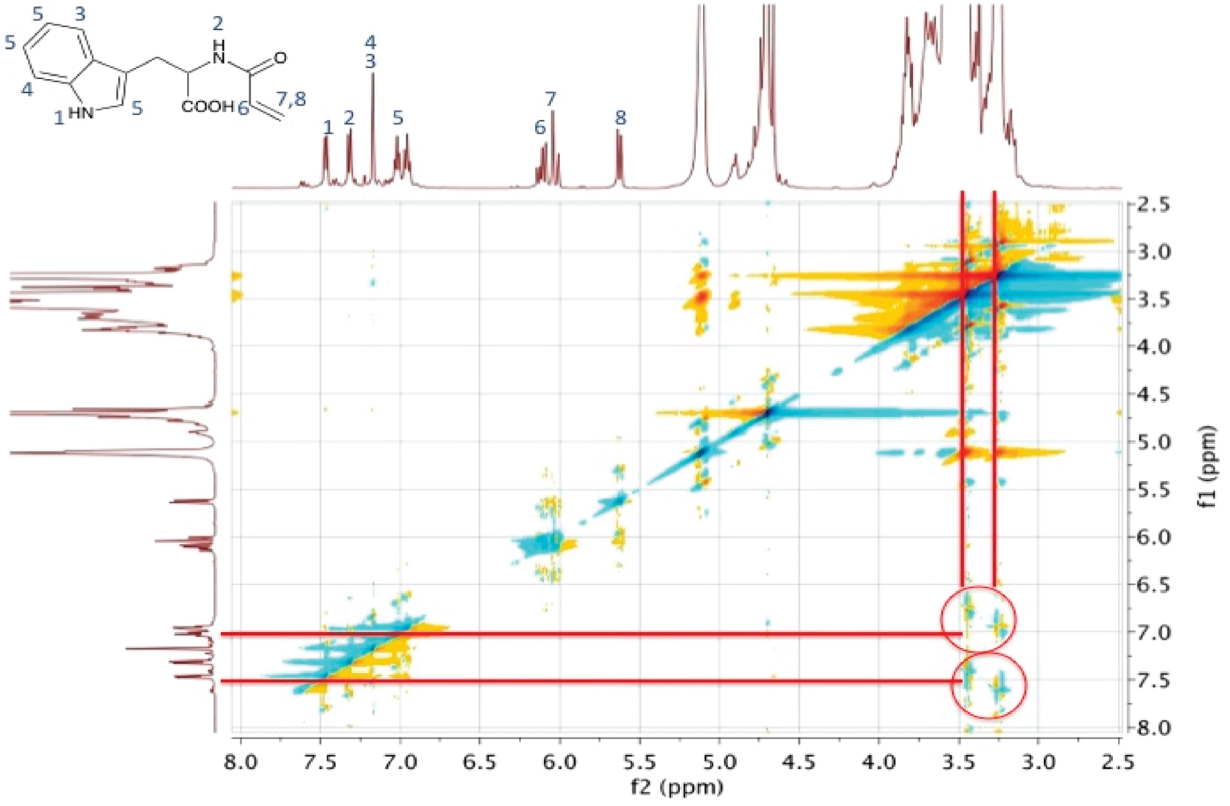
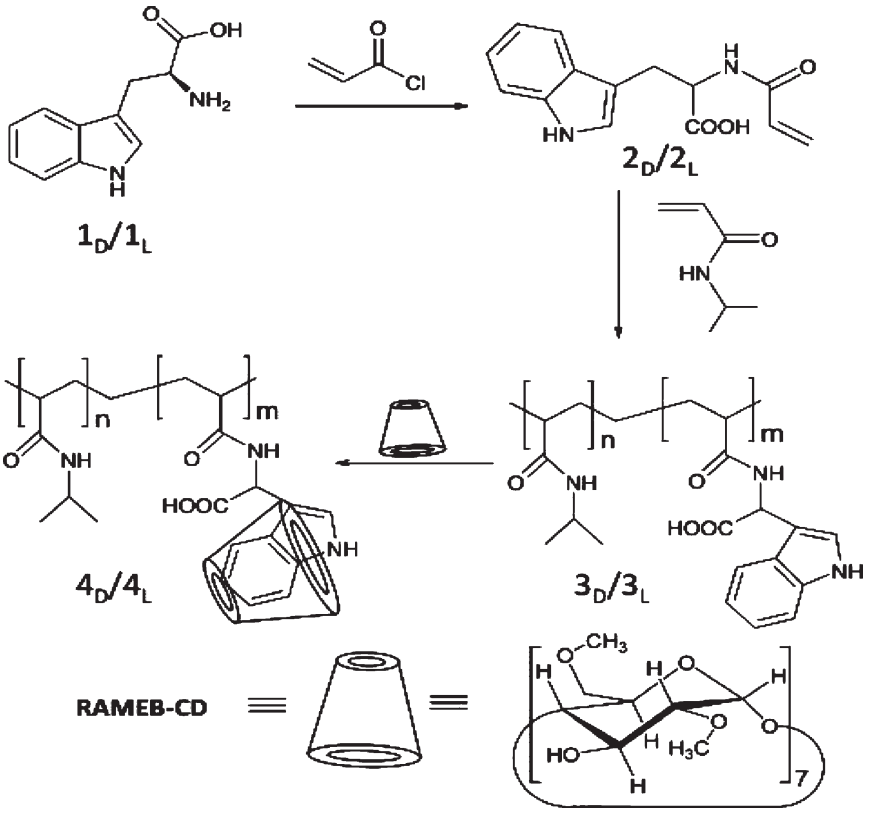



**Figure 1.** 2D ROESY NMR of $\mathbf{2_D}$ with DIMEB-CD.

**Scheme 1. Synthetic Pathway to the Chiral pH- and Temperature-Responsive Copolymers $3_D$ and $3_L$ and Correspondingly Their Supramolecular Systems $4_D$ and $4_L$ with RAMEB-CD**




Thus, turbidity measurements showed that the cloud points (LCST) of the all copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ are almost identical for both enantiomers and only influenced by contribution of Trp moieties in the copolymer structure. As we expected, it results in a

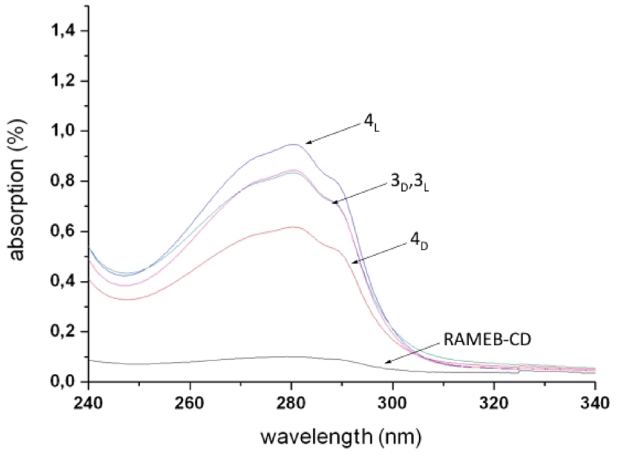



**Figure 2.** UV spectrum of the RAMEB-CD copolymers (1:20) $\mathbf{3_{D,L}}$ and $\mathbf{4_{D,L}}$ in aqueous media (0.5 mg/mL, pH 6).

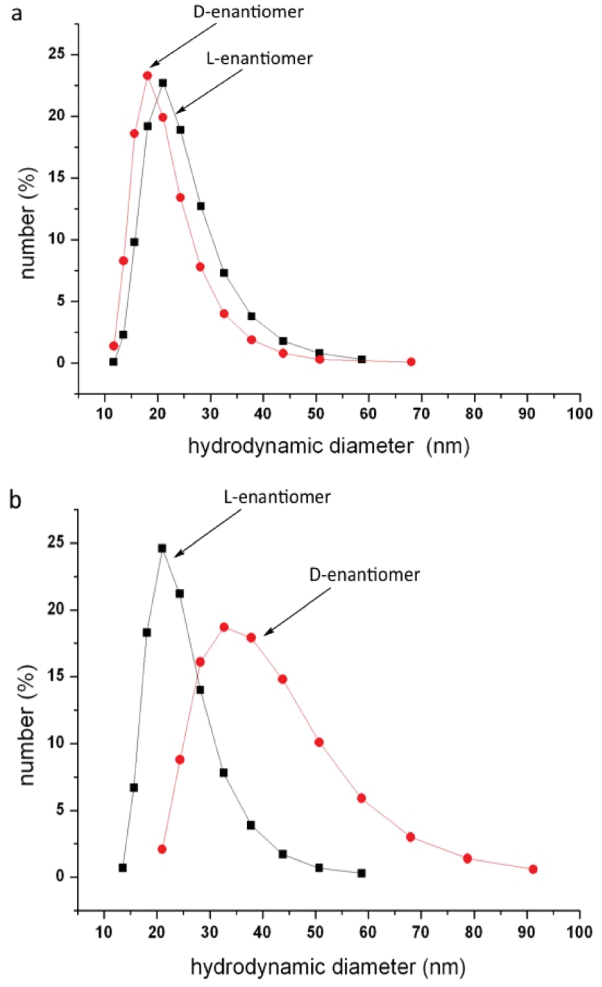



**Figure 3.** Hydrodynamic diameter: (a) copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ (1:15); (b) correspondingly after complexation with RAMEB-CD (1 mg/mL, pH 6).

significant decrease in the LCST value and even poor water solubility of the copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ (1:10) (Supporting Information).

Because of the presence of free carboxylic groups, the turbidity is affected by changes in the pH value. For example, at pH of 2−4 the protonated copolymers $\mathbf{3_D}$ and $\mathbf{3_L}$ (1:20) showed a relatively low LCST at around 22 °C compared to a LCST above 28 °C at high pH values around 12 (Supporting Information). This phenomenon is caused by the conversion of the −COOH to the negatively charged state of $-COO^-$ under base conditions. The carboxylic groups and the high hydrophilicity may efficiently prevent the aggregation of the NIPAAm polymer chains, thus leading to better solubility in water. According to IR spectroscopy at a low pH the majority of the acid groups are protonated (1720 $cm^{-1}$) (Supporting Information). Since the carboxyl groups are deprotonated at high pH values, the characteristic for carboxylic group peak vanishes. Attributable to enantioselective complexation of RAMEB-CD with the Trp aromatic side groups, the phase transition temperatures for the ($\mathbf{4_{D,L}}$) copolymers are shifted and are different for both enantiomers in value. It turned out that the attached D-enantiomer interacts more conclusively with RAMEB-CD. Correspondingly, the LCST increased a few degrees higher after RAMEB-CD addition compared to the L-enantiomer (Supporting Information). This temperature-dependent solubility was a fully reversible process.

Additionally, by means of the DLS technique, the hydrodynamic diameters of the copolymers can be detected relatively easily. Evidently, the $\mathbf{3_D}$ and $\mathbf{3_L}$ copolymers possess nearly identical mean coil sizes. Since the RAMEB-CD is added to the copolymer solution, hydrodynamic diameters are affected dramatically with remarkable definition for both enantiomers. As an example, in Figure 3 the variation in the average particle hydrodiameter ($D_h$) of $\mathbf{3_{D,L}}$ (1:15) (Figure 3a) compared to the $\mathbf{4_{D,L}}$ (1:15) (Figure 3b) is plotted together. The polymer bearing the D-enantiomer shows a larger increase (32 nm) in the coil dimension in comparison with the less stable L-enantiomer complex (24 nm) under identical experimental conditions (Supporting Information).

It can be concluded that the results indicate the possibility of enantioselective recognition of the polymeric attached chiral Trp amino acid in a supramolecular environmental response model system. In this work, we have succeeded in enantiospecific recognition using UV spectroscopy, DLS technique, and turbidity method. Thus, our findings can generate a new model for chirality identification of side groups with RAMEB-CD in polymer matrixes, which can potentially offer great opportunities, e.g., in drug development.

**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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