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MOLECULAR IMPRINTING OF CYCLODEXTRIN IN HOMOGENEOUS SOLUTIONS

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In homogeneous and alkaline aqueous solutions, cyclodextrin was reacted with epichlorohydrin in the presence of various templates. The molecularly imprinted polymers, which involve 2-4 cyclodextrin residues and are completely soluble in water, satisfactorily recogndasdasdaized the template molecules and selectively bound them in water. New methodology to prepare receptors for nanometer-scaled guests in a tailor-made fashion has been developed.

В однородных и щелочных водных растворах циклодекстрин вступает в реакцию с эпихлоргидрином в присутствии разных темплетов (эталонов). Молекулярно распечатанные полимеры, которые включают 2-4 остатка циклодекстринов и полностью растворимы в воде, соответственно узнают молекулы темплета и избирательно связывают их в воде. Разработана новая методология приготовления рецепторов в виде "мужского" покроя для нанометр-масштабированных "гостей".

Սիատարր և հիմնային ջրային լուծույթներում ցիկլոդեքստրինը ռեակցիայի մեջ է մտնում էպիքլորհիդրինի հետ տարբեր թեմփլեթների (էտալոնների) ներկայությամբ: Մոլեկուլյար բացված պոլիմերները, որոնք ներառում են ցիկլոդեքստրինի 2-4 մնացորդներ և լիովին լուծելի են ջրում, համապատասխանաբար ճանաչում են թեմփլեթի մոլեկուլները և ընտրողաբար կապում են դրանք ջրի մեջ: Նանոմետր-մասշտաբային «հյուրերի» համար մշակվել է ռեցեպտորների պատրաստման նոր մեթոդոլոգիա տղամարդու ձևածքի տեսքով.

Introduction

Host-guest chemistry has made such a great progress that we can now prepare very good receptors rather easily and successfully as long as the target guest is small (e.g., the diameter < 5 Å) [1]. Cyclodextrins (CDs) and their derivatives are typical examples [2]. However, the design of receptors for large guest molecules (10 Å diameter or greater) has not yet been very successful. Thus there still remains a big gap between naturally occurring receptors and artificial ones [3]. These receptors are regarded as the key for the future science and technology, in which wide spectrum of large molecules must be precisely differentiated from each other. Recently, we presented a new methodology for the preparation of artificial receptors which selectively and efficiently bind nanometer-scaled guests in water [3-6]. By using molecular-imprinting technique [7], CD molecules are built up in a predetermined way (Figure 1). Each of the CD molecules in the ordered assembly binds the predetermined portion of the target guest, and thus the assembly as a whole recognizes this guest very exclusively [8].

Figure 1. Molecular-imprinting of CD to prepare polymeric receptors for nanometer-scaled guests.

As described previously [3-6], the molecular imprinting of CDs (their crosslinking) was achieved either in DMSO or in water in the presence of the target guest. By these procedures, receptors for steroids [4], peptides [5], and antibiotics [6] were successfully prepared. In the cholesterol receptors, for example, the binding-sites are formed from two CD molecules which are connected at the secondary hydroxyl sides [4]. The regulation of mutual conformation of CDs by the molecular-imprinting effect has been concretely evidenced by MALDI-TOFMS. Under the imprinting conditions, the template places two or more CD molecules in a close proximity and accelerates the crosslinking between them. These arguments were supported by the NMR analysis.

As described above, the molecular-imprinting of CD can provide very selective receptors for many nanometer-scaled guests in a tailor-made fashion, and thus is highly promising for future applications. The receptors obtained were highly crosslinked polymers which are useful for practical purposes. However, water-soluble receptors are also useful for applications, although it has not yet been clear if the polymeric receptors must be water-insoluble or not. Another question is if the molecular-imprinting must be achieved in heterogeneous systems, as usually done before, or it is also accomplished in homogeneous mixtures Information on these points is important for further developments.

In this paper, we show that the molecular imprinting of CD can be successfully achieved even in homogeneous solutions. The CD polymers as tailor-made receptors for nanometer-scaled guests are obtained either in homogeneous states or in heterogeneous states. One can easily choose either of these two methods, depending on the purpose. This finding should greatly widen the scope of application of this novel methodology.

Materials and Methods

β-Cyclodextrin (β-CD) and other chemicals were commercially obtained. Molecular imprinting of β-CD was achieved by reacting β-CD with epichlorohydrin in aqueous NaOH solutions in the presence of appropriate template. Typical reaction conditions for the molecular imprinting were as follows: [β-CD] = 0.2 M, [epichlorohydrin] = 1.4 M, [NaOH] = 2 M, and [the template] =0.1 M. After the reactions at 25°C for 8 h, the mixtures were dialyzed with water for 3 days by using a seamless cellulose membrane (UC 16-32-100 (Sanko Junyaku Co., Ltd.)). Complete removal of the template, the crosslinking agent, and others from the polymers by this procedure was confirmed by using UV-visible absorption spectroscopy. Circular dichromism (CD) spectra were obtained at pH 8 (50 mM phosphate buffer) on a Jasco J-725 spectropolarimeter. MALDI-TOFMS was run on a Shimadzu/KRATOS KOMPACT TYPE I spectrometer.

Results and Discussion

When L-Phe-L-Phe was used as the template, the molecular imprinting of β-CD under the above conditions proceeded in completely homogeneous solutions (see Figure 1). On the other hand, with the use of Z-Trp-Phe as the template, the reaction mixtures were heterogeneous in the early stage of the reactions. However, the mixtures gradually became homogeneous as the reaction proceeded, and totally homogeneous solutions were obtained at the end of the reactions. According to MALDI-TOFMS spectroscopy, the products, obtained after the dialysis with a cellulose membrane (see the Materials and Methods section), are composed of monomeric, dimeric, trimeric, and tetrameric β-CDs, in which the corresponding number of β-CD molecules are connected by epichlorohydrin-derived residues. Furthermore, several 2,3-dihydroxyethyl residues are bound to these β-CD oligomers. These residues come from the epichlorohydrin molecules, which react with the hydroxyl group of one β-CD molecule and the other ends react with water molecules (or hydroxide ion) instead of another β-CD molecule. As expected, the degree of substitution of β-CD by the 2,3-dihydroxyethyl residues showed a normal distribution. In these homogeneous solutions, β-CD molecules were efficiently crosslinked with each other by epichlorohydrin, and totally water-soluble polymeric receptors towards the template molecules have been successfully obtained.

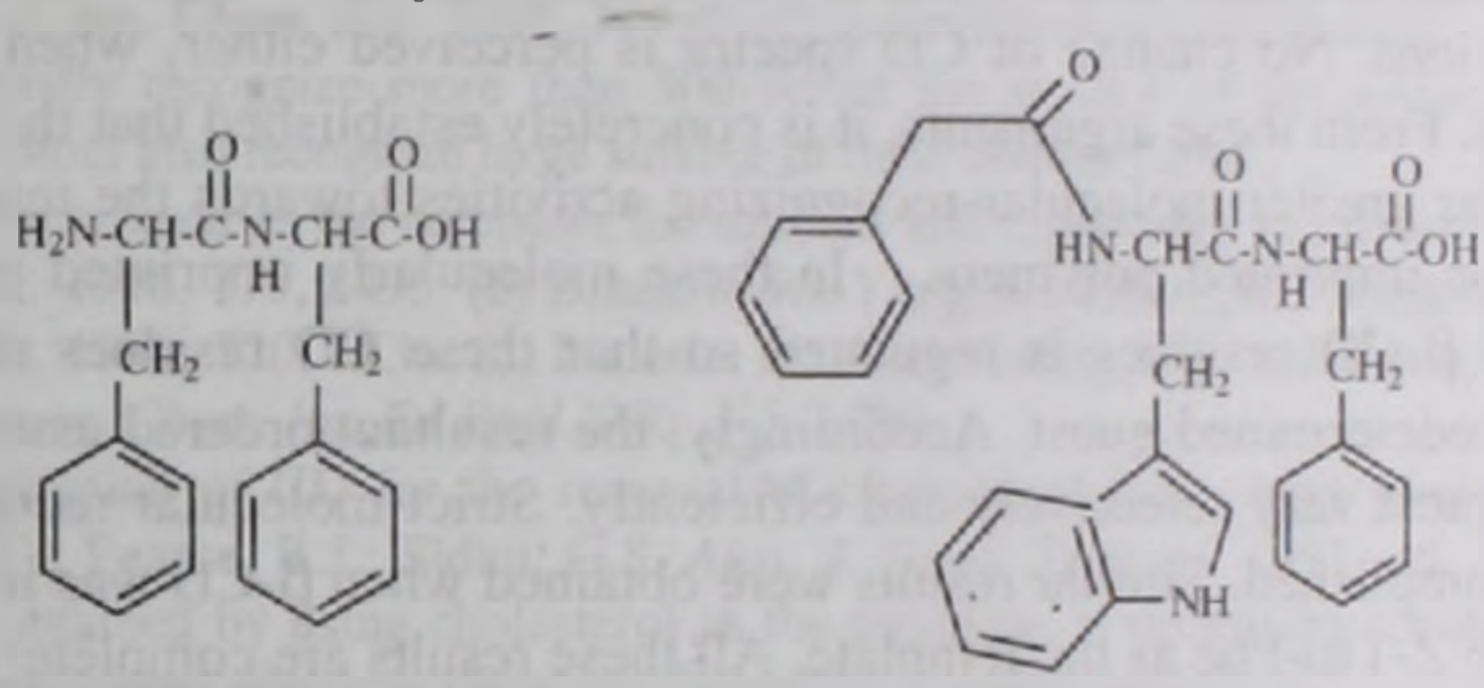

Figure 2. CD spectra of L-Phe-L-Phe in the presence of (A) the imprinted β-CD polymer obtained by using L-Phe-L-Phe as the template and (B) non-imprinted β-CD polymer: the concentrations of β-CD residue in the polymer are (a) 0.0, (b) 1.0, (c) 2.0, (d) 5.0, and (e) 10.0 mM. [L-Phe-L-Phe] = 0.9 mM at pH 8 (phosphate buffer).

Figure 2 (A) shows the CD spectra of L-Phe-L-Phe (guest) in the pH 8 solutions which contain the water-soluble imprinted β-CD polymers. The polymers were prepared by using L-Phe-L-Phe as template (in this case, L-Phe-L-Phe is used as both the template and the guest). As the amount of the imprinted β-CD polymer in the solutions is increased, the CD signal due to the L-Phe-L-Phe gradually and monotonically shifts towards longer wavelength. Apparently, L-Phe-L-Phe is clearly recognized by the imprinted β-CD polymer. In contrast, only marginal change of CD spectrum is observed when D-Phe-D-Phe is used as the guest in place of L-Phe-L-Phe. Thus, the induced CD is specific to L-Phe-L-Phe as the guest (note that this polymer was obtained by using L-Phe-L-Phe as the template). The present imprinted β-CD polymers exhibit sufficient enantio-selectivity.

In contrast with the efficient molecular recognition of L-Phe-L-Phe by the L-Phe-L-Phe-imprinted β-CD polymers, non-imprinted β-CD polymers, prepared by crosslinking β-CD in the absence of the template under the same conditions, show no changes in the CD spectra of L-Phe-L-Phe (Figure 2 (B)). These non-imprinted polymers are also composed of monomeric, dimeric, trimeric, and tetrameric β-CDs. Even at higher concentrations of β-CD residue in the polymer, the change of spectrum is only marginal. Furthermore, the parent β-CD itself (in the monomer form) does not induce any change in the CD spectra, when it is mixed with L-Phe-L-Phe in the solutions of pH 8. The formation constant of the β-CD/L-Phe-L-Phe complex is rather small (around 10 M^{-1}) so that this complex is hardly formed in the sample solutions. No change of CD spectra is perceived either, when D-Phe-D-Phe is used as the guest. From these arguments, it is concretely established that the imprinted β-CD polymers have far greater molecular-recognizing activities towards the template molecules than those of non-imprinted polymers. In these molecularly imprinted polymers, mutual conformation of β-CD residues is regulated so that these CD residues show cooperative binding to the predetermined guest. Accordingly, the resultant ordered assemblies of β-CD bind the target guest very selectively and efficiently. Strict molecular recognition has been successfully accomplished. Similar results were obtained when β-CD was imprinted towards another dipeptide Z-Trp-Phe as the template. All these results are completely consistent with our previous results on water-insoluble β-CD polymers as tailor-made receptors[3-6].

In conclusion, the present study has confirmed the following two facts. (1) The molecular imprinting of β-CD is successfully achieved even in homogeneous solutions, as well as in heterogeneous mixtures. (2) Homogenous receptors for nanometer-scaled guests can be obtained in a tailor-made fashion by imprinting β-CD towards the target guest. Both of these factors further substantiate that the molecular imprinting of CD is one of the most promising methods to prepare artificial receptors for large guest molecules, and that this method is applicable to versatile guests. They are certainly applicable to separation of desired products, removal of impurities, and other purposes in chemical industry and biotechnology [10]. Preparation of artificial antibodies and artificial catalytic antibodies by the molecular imprinting are certainly the next targets. These attempts are currently under way in our laboratory.

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