Carbohydrate Polymers **10** (1989) 183–188

The Synthesis of Water-Soluble Polymers of β-cyclodextrin and their use in Aqueous Two-Phase Systems

Björn Ekberg, Börje Sellergren, Lisabeth Olsson & Klaus Mosbach*

Department of Pure and Applied Biochemistry, Lund Institute of Technology, University of Lund, PO Box 124, S-221 00 Lund, Sweden

(Received 19 June 1988; accepted 11 August 1988)

ABSTRACT

A highly water-soluble β-cyclodextrin polymer was prepared from β-cyclodextrin by crosslinking with epichlorhydrin under basic conditions. This polymer could, unlike β-cyclodextrin itself, be partitioned to one phase in aqueous two-phase systems. The binding of some organic compounds to the polymer in such systems is demonstrated.

INTRODUCTION

Cyclodextrins and their chemistry have been the subject of extensive investigations during a number of years (Bender & Komiyama, 1978). These are cyclic oligosaccharides with a central hydrophobic cavity surrounded by a hydrophilic outer shell. Since they can selectively bind different compounds which are complementary to the cavity in size and hydrophobicity, they have been used for various separation purposes. Covalently linked to a solid phase, they have been used for the separation of racemic mixtures, barbiturate derivatives, dansylaminoacids and aminoacid–naphthylamides (Armstrong & DeMond, 1984). Furthermore they have been used for the separation of isomers of naphthylamine (Fujimura *et al.*, 1983), *cis/trans* cyclohexane derivatives (compounds of importance for the physical properties of polyester polymers, Tindall, 1987), and they have found wide applications in the

*To whom correspondence should be addressed.

Carbohydrate Polymers 0144-8617/89/$03.50

removal of undesired compounds from fruit juices and removal of caffeine from aqueous solutions (Shaw & Buslig, 1986).

The low load capacity of the solid β-cyclodextrin phases focused our attention on the use of a soluble β-cyclodextrin polymer (β-CDP) in aqueous two-phase systems. This we believed would increase the sample load capacity due to the rather high solubility of β-cyclodextrin polymers compared to β-cyclodextrin monomers. Moreover, partitioning in aqueous two-phase systems has proved to be a valuable preparative technique for purifying cell particles and biomolecules (Albertsson, 1971) and for preparative racemic resolution of organic compounds (Ekberg *et al.*, 1985; Sellergren *et al.*, 1988 *a, b*).

MATERIALS AND METHODS

Chemicals

Dextran T-10, Dextran T-40 and dextransulfate (molecular weight 50000) came from Pharmacia (Uppsala, Sweden). Polyethylene-glycol (PEG)-6000 and PEG-20000 were obtained from Union Carbide (New York, USA). Epichlorhydrin was obtained from Sigma (St Louis, USA), L-(U-^{14}C)-Alanine 174 mCi mmole^{-1} from Amersham International (UK). Tresylchloride came from Fluka AG (Switzerland), dansylchloride from BDH Chemicals (Poole, UK) and the cyclohexylphenyl acetic acid was purchased from Janssen Chimica (Beerse, Belgium). The β-cyclodextrin used was a gift from Stadex AB (Malmö, Sweden). R,S-terbutaline (R,S,-5-[2-(t-butyl)amino-1-hydroxy-ethyl]-1,3-benzenediol) was provided by Draco AB (Lund, Sweden).

Synthesis

D,L-Dansylphenylalanine was prepared by reacting the amino acid with dansylchloride in tetrahydrofuran–water in the presence of triethylamine. Purification was by preparative TLC. The β-cyclodextrin polymer (β-CDP) was prepared by crosslinking β-cyclodextrin under strongly alkaline conditions with epichlorhydrin (Fenyvesi *et al.*, 1981): 10·5 g of β-cyclodextrin was dissolved in 25 ml of 35% NaOH. Epichlorhydrine (8·5 ml) was added in small portions and the reaction solution was heated to 90°C for 5 min. After cooling the solution was neutralized with 6 M HCl and subjected to dialysis (molecular weight cut-off, 3500). Lyophilization yielded 8 g of highly water soluble (60%, w/w) β-CDP as

compared to the relatively low solubility (2%, w/w) of the β-CD monomer.

The ^{14}C-labeled β-CDP was prepared by tresylchloride activation (Nilsson & Mosbach, 1981) of the hydroxyl-groups; 2·5 g of β-CDP in 17 ml of dry acetone was followed by the addition of 40 μl of dry pyridine and 10 μl of tresylchloride. Coupling was performed with 12·5 μCi of ^{14}C-alanine at pH 8·0. After termination with Tris-buffer at pH 8·5, the labeled polymer was purified by extensive dialysis.

Analysis

HPLC-analysis of the dansylphenylalanine-containing samples was carried out on LKB 2150-52 equipment with a β-CD column (4 × 250 mm, packed with 5 μm β-CD silica). The mobile phase consisted of methanol/(0·05 M ammonium acetate pH 6·0):1/1 and the elution was monitored at 254 nm.

HPLC-analysis of the terbutaline-containing samples was performed on the LKB equipment with an RP-18 column (4 × 100 mm). The mobile phase was 2% (w/w) β-CD in methanol/(25 mM citrate pH 6·0):5/95, and the elution was monitored at 260 nm.

The distribution of the β-CDP was determined by polarimetric measurements on a Perkin–Elmer model 141 polarimeter except for system 5 where the distribution was determined by the addition of labeled β-CDP.

Distribution of the compounds

10 μmoles of each substance were incubated with 1 g of phase system overnight at room temperature. The distributions of D,L-phenylanine (Phe), R,S-cyclohexylphenyl acetic acid (CHPAA) and D,L-tryptophane (Trp) in the phase systems were determined by comparing the absorbance at 257 nm (Phe and CHPAA) and at 280 nm (Trp), of the upper and lower phase. The distribution of terbutaline and dansylphenylalanine was instead determined by HPLC-analysis (see above).

RESULTS AND DISCUSSION

The β-CDP used in this study had a molecular weight of more than 3500 (upper limit not determined) and could be dissolved up to 60% (w/w) in water. The distribution of the β-CDP in a number of currently used aqueous two-phase systems was studied. Unlike β-cyclodextrin itself, a significant partitioning was observed for the β-CDP in the systems listed

TABLE 1
Distribution of β-CDP in Various Aqueous Two-phase Systems

System	*% of β-CDP in upper phase*
(1) PEG-6000 7%/Dextran T-10 10%, β-CDP 11%	68
(2) PEG-6000 10%/Dextran T-40 10%, β-CDP 11%	61
(3) PEG-20000 10%/Dextran T-10 10%, β-CDP 11%	70
(4) PEG-6000 7%/Dextransulfate 10%, 0·3 M NaCl, β-CDP 11%	77
(5) β-CDP 20%/Dextransulfate 10%, 0·3 M NaCl	86

TABLE 2
Distribution of Various Organic Compounds in System 4, in the Presence or Absence of β-CDP and in System 5

Substance	*System*	*% in upper phase*
Phe	4	66
CHPAA	4	90
DansylPhe	4	89
Trp	4	65
Terbutaline	4	68
Phe	4, without β-CDP	53
CHPAA	4, without β-CDP	78[a]
DansylPhe	4, without β-CDP	79
Trp	4, without β-CDP	64
Terbutaline	4, without β-CDP	60
DansylPhe	5	66
Terbutaline	5	90

Phe = phenylalanine; CHPAA = cyclohexylphenyl acetic acid; DansylPhe = dansylphenylalanine; Trp = tryptophane.
[a]Only partly dissolved.

in Table 1. The β-CDP did not behave as biomolecules and particles in the studied systems allowing the possibility of directing the distribution to one phase by increasing the molecular weight of the other phase (Albertsson, 1971). Nor was it possible to alter the distribution by changing the concentration of the polymers. However, 77% of the added β-CDP was found in the upper phase in a PEG–dextransulfate system and 86% of the β-CDP was found in the upper phase in a β-CDP–dextransulfate system. Hence systems 4 and 5 were chosen for the further studies.

In order to investigate whether the β-CDP possessed similar binding characteristics as β-CD monomers the distribution of a few organic compounds in system 4 with and without β-CDP and in system 5 was determined (Table 2). The solubilizing property of the β-CDP was particularly apparent in the case of the increased solubility of cyclohexylphenyl acetic acid in system 4 as compared to its limited solubility in system 4 without the β-CDP. Considering the other compounds they were bound to a varying degree resulting in a significant enrichment of the compounds in the upper phase.

TABLE 3
Distribution of Dansyl-phenylalanine in System 5 at Various pH-values

pH	*% in upper phase*
6·7	66
4·7	73
3·4	77

Since we assume that the binding to the β-CDP is controlled by hydrophobic interactions the binding of dansyl-phenylalanine to the β-CDP should exhibit pH-dependence. This was therefore investigated (Table 3). When lowering the pH-value the hydrophobic interactions are expected to become more important resulting in a stronger binding and thus in an increased enrichment in the upper phase, which indeed was observed.

Due to the observed binding characteristics, the simple synthesis and the inexpensive raw materials, we believe that the highly water-soluble cyclodextrinpolymers prepared in this study might find important applications in aqueous two-phase systems for the large scale separation of cyclodextrin binding compounds.

ACKNOWLEDGEMENTS

We wish to thank Mrs Agneta Walhagen, Department of Technical Analytical Chemistry, University of Lund, for the preparation of the β-cyclodextrin column. The generous gift of cyclodextrin from Stadex AB (AVEBE) Malmö, Sweden, is gratefully acknowledged. This work was supported by the Swedish Board for Technical Development.

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