Cyclodextrins in Polymer Chemistry: Enzymatically Catalyzed Oxidative Polymerization of Para-Functionalized Phenol Derivatives in Aqueous Medium by Use of Horseradish Peroxidase

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ABSTRACT: Ethyl 1-[(4-hydroxyphenyl)aminocarbonyl)]-2-vinylcyclopropane carboxylate has been oligomerized using horseradisch peroxidase as catalyst. The oligomerization was achieved in the presence of cyclodextrine at room temperature in phosphate buffer (pH 7). The oligomer formed was cross-linked via free radical polymerization.

Introduction

The oxido-reductase horseradish peroxidase (HRP) catalyzes oxidative oligomerization of various electron-rich aromatic molecules like ortho- and meta-substituted phenol derivatives. This is a potential alternative method for preparation of phenol resins without using toxic formaldehyde. The structure and catalysis mechanism of HRP have been intensively studied.[1−4]

Because of the poor solubility of phenol derivatives, the HRP-catalyzed oligomerizations are often carried out in a mixture of aqueous buffer and organic solvent, such as dioxane or acetone. In the mixtures of acetone and water (pH 6), Liu et al. have achieved the polymerization of azophenol derivatives with functional groups like CN−, CH_3O−, NO_2−, SO_3−, and COO− at the para-position.[5]

In recent studies we have reported that many organic monomers form soluble host−guest complexes with 2,6-di-*O*-methyl-β-cyclodextrin (RAMEB), which can be polymerized in water.[6] In this connection the enzymatic polymerizations of para- and meta-substituted phenols in water were achieved, in which RAMEB was used as host to form soluble complexes with the hydrophobic phenol monomers.[7] A polymerization degree of at least 6−10 with a yield up to about 100% was achieved through the enzymatic polymerization of these complexes in water (pH = 7). In contrast, in the absence of RAMEB, only very low yields of these oligophenols were obtained.

The enzymatic polymerizations of phenol derivatives bearing a furamide group, maleimide group, and nitrone group at the para-position were also studied. These copolymers were cross-linked via thermal cycloaddition reactions.[8−10]

Vinylcyclopropanes (VCP) are interesting monomers because of low volume shrinkage during the radical polymerization. 1,1-Disubstituted VCPs undergo radically 1,5-ring-opening polymerization when the substituents are capable of stabilizing the radical formation.[11−13] The lower volume shrinkage exhibited by VCPs is a consequence of ring-opening polymerization.[14] In recent years many new VCP derivatives have been investigated.[15−19] But the VCP derivatives attached with phenol have not been studied. In this paper we report the synthesis and polymerization of VCP derivatives attached with phenol, which are potential candidates for dental materials.

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Results and Discussion

The preparation of ethyl 1-{[(4-hydroxyphenyl)amino]-carbonyl}-2-vinylcyclopropanecarboxylate (**5**) was carried out by condensation of 1-(ethoxycarbonyl)-2-vinylcyclopropanecarboxylic acid (**1**) with 4-aminophenol (**2**) (Scheme 1). Because the aromatic amino group of 4-aminophenol is relatively inert compared to the aromatic hydroxyl group, 4-aminophenol was at first modified with trimethylsilyl chloride, so that the hydroxyl group is protected and the amino group is activated. The formed derivative **4** reacted selectively with the acid chloride **3** to an intermediate, which at the next step was hydrolyzed to **5**.

The water-soluble complex **6** was prepared simply by stirring a dispersion of the monomer and RAMEB in water (1:1) at room temperature. Then water-soluble HRP was added, and oligomerization was initiated by H_2O_2. The clear solution of the monomer complex **6** became immediately turbid after addition of the first drop of H_2O_2. The formed oligomer is insoluble in water but in some organic solvents, such as chloroform, acetone, ethanol, THF, and DMF. The vinyl group of the formed oligomer **7** was not affected under these polymerization conditions and thus would be used to cross-link the oligomers via free radical polymerization in the presence of AIBN (Scheme 2).

The formation of complex **6** was verified by ^{1}H NMR spectra (Figure 1). In comparison to the free monomer **5**, the chemical shifts of signals of the cyclopropane ring and the vinyl group in the host−guest complex **6** were significantly influenced (δ from 2.50 to 2.42 ppm, *e* and *f* from 1.78 to 2.03 ppm and from 1.64 to 1.95 ppm, *c* from 5.67 to 5.76 ppm, *a* and *b* from 5.19 to 5.26 ppm and 5.35 to 5.39 ppm). This indicates that the RAMEB forms an inclusion complex with **5**, in which the vinylcyclopropane group is preferably enclosed in the hydrophobic cavity of RAMEB.

The molecular weight of **7** was estimated by MALDI−TOF MS (Figure 2, with addition of K^+). For a better detection of higher molecular fractions kalium salt was added. The peaks show exactly the molecular masses of the oligomers plus kalium, e.g., the octamer peak at

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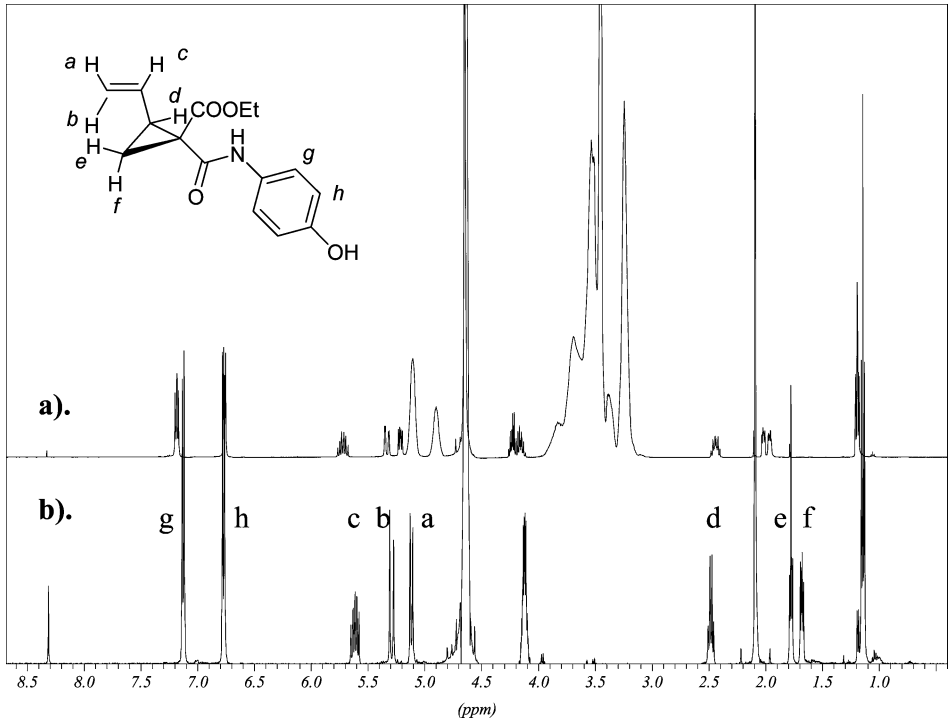
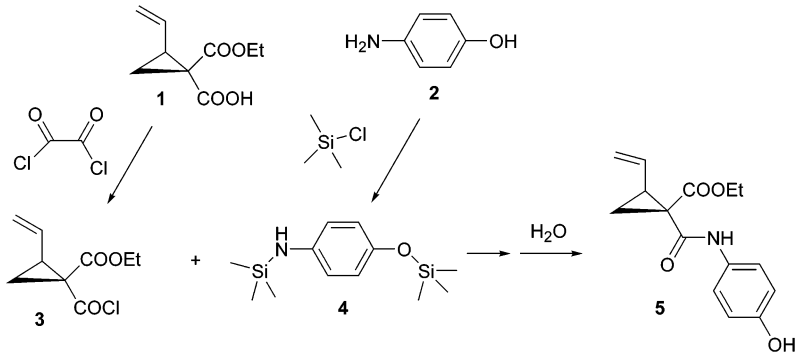
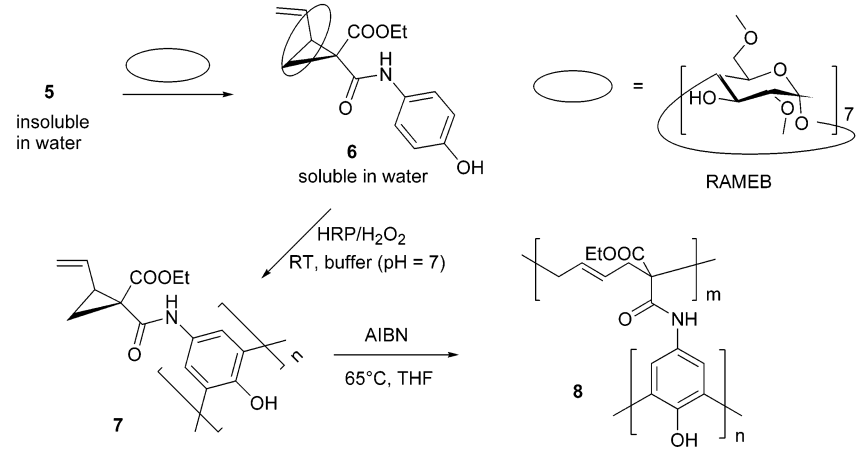


Figure 1. ^{1}H NMR spectra of (a) complex **6** and (b) monomer **5** (500 MHz, D_2O).

Scheme 1. Preparation of the Monomer



Scheme 2. Oligomerization of Vinylcyclopropane Derivatives



2773.5 u/e = 273.3 × 10 + 2 + 39 (K^+). The repeat unit corresponds the mass of a monomer unit, e.g., 1953.5 − 1680.4 = 273.1.

The structures of polyphenols formed via enzymetic polymerization were already discussed in several papers. It was confirmed that those oligomer are composed of either phenylene units[6,8,9] or oxyphenylene units.[7]

In this work, the structure of the formed oligomer **7** was verified by ^{1}H NMR (Figure 3) and FT-IR spectroscopy.

Figure 3a shows the broad signals of a typical polymer. The signals a, a′, b, c, c′, and d indicate that the cyclopropane ring and vinyl group are undamaged incorporated into the formed oligomer.

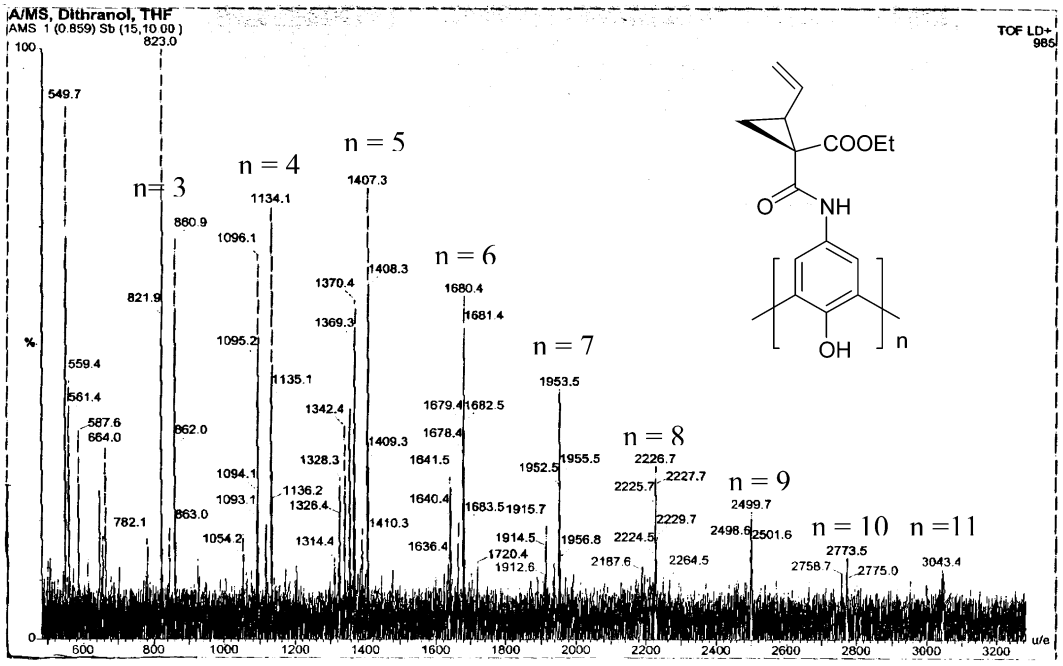


Figure 2. MALDI−TOF spectrum of oligomer **7**.

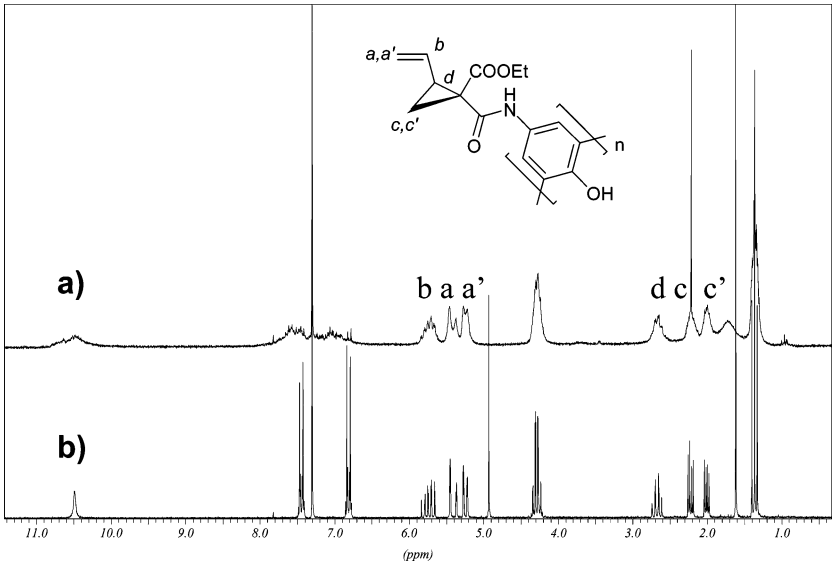


Figure 3. ^{1}H NMR spectra of (a) oligomer **7** and (b) monomer **5** (200 MHz, $CDCl_3$).

A comparison of the FT-IR spectrum with that of the monomer demonstrates that a new ether band does not appear in the range 1080−1280 cm^{-1} after polymerization. Furthermore, the band at 1339 cm^{-1} suggests the existence of the OH group in the oligomer. The typical band at 870 cm^{-1} indicates a benzene ring with an isolated H atom, e.g., 1,3,4,5-tetrasubsituted bezene ring. As described above, the FT-IR spectra prove that the formed oligomer is composed via directly linked phenylene units.

Free radical polymerization of the formed oligomer **7** using AIBN as initiator at 65 °C leads to formation of a network. The cyclopropane ring is opened during the radical polymerization. The signals at 974 cm^{-1} in the FT-IR spectrum of the cross-linked polymer **8** are assigned to the trans vinyl group. The network **8** is a pale orange hard powder that is insoluble in general organic solvents like DMSO, DMF, or THF.

Conclusion

2,6-Di-*O*-methyl-*β*-cyclodextrin forms a hydrophilic host−guest complex with the hydrophobic monomer **5**. At room temperature, horseradish peroxidase acts as an effective catalyst and hydrogen peroxide as an effective initiator for oligomerization. Cyclodextrin can be easily removed by washing with a mixture of acetone and water. The formed oligomer is soluble in general organic solvents and can be radically cross-linked. The obtained polymer is relatively thermally stable.

Experimental Section

Materials. HRP (1044 U/mg) was purchased from Bio-Chemika. 1,1-Diethoxycarbonyl-2-vinylcyclopropane was provided by Ivocar-Vivadent (Schaan, EU). RAMEB (degree of methylation 1.8) was purchased from Wacker-Chemie. The other reagents were purchased either from Fluka or from Merck and used as received. The solvents with p.a. quality were stored over molecular sieves of 3 or 4 Å. The technical

solvents for flash column chromatography were distilled before usage.

Measurements. The 200 MHz ^{1}H NMR spectra were measured on a Bruker Avance DRX 200, IR spectra were recorded with a Nicolet 5SXB FTIR spectrometer, and MALDI−TOF spectra were measured with a Micromass TofSpec-MS spectrometer. DSC measurements were carried out with a Mettler DSC 30, and the melting points were measured with a Büchi 510.

Synthesis of 3. 1-(Ethoxycarbonyl)-2-vinylcyclopropanecarboxylic acid (**1**) was prepared from 1,1-diethoxycarbonyl-2-vinylcyclopropane in 70% yield in accordance with the procedure reported earlier.[20]

7.62 g (60 mmol) of oxalyl chloride was slowly dropped to a solution of 7.95 g (43 mmol) of **1** in 20 mL of dichloromethane, which was placed in a three-necked flask and cooled in an ice−water bath. The mixture was then heated at 40 °C for 4 h. The solvent and unreacted oxalyl chloride were removed under reduced pressure (ca. 30 mbar). The residual acid chloride (**3**) was used directly for the following preparation of **5**.

13.04 g (120 mmol) of trimethylsilyl chloride was added dropwise at 5 °C to a solution of 4.70 g (43 mmol) of 4-aminophenol and 12.14 g (120 mmol) of *N*,*N*,*N*-triethylamine in 200 mL. The mixture was heated and refluxed for 2 h, and then the solution of crude **3** in some dichloromethane was added. Subsequently, the solution was stirred at room temperature (RT) for 20 h, the formed precipitate was removed, and the solution was hydrolyzed by stirring with saturated $NaHCO_3$ solution. The collected organic phase was hydrolyzed again with water (pH 5−6). The product was collected as usual and purified by flash column chromatography using acetone/$CHCl_3$ (1:8 v/v) to give 7.57 g of colorless crystalline solid **5**.

Yield (in comparison with **1**) 64%, colorless crystalline solid, mp 85−86 °C.

^{1}H NMR (200 MHz, $CDCl_3$): δ 10.48 (br s, NH), 7.42 (ddd, 2H), 6.80 (ddd, 2H), 5.74 (m, 1H), 5.41 (m, 1H), 5.25 (m, 1H), 4.29 (q, 2H), 2.68 (m, 1H), 2.22 (m, 1H), 2.01 (m, 1H), 1.36 (t, 3H).

^{13}C NMR (500 MHz, $CDCl_3$): δ 172. 82, 167.38, 153.78, 134.14, 131.97, 123.52, 121.14, 116.79, 62.86, 39.04, 35.69, 22.77, 15.31.

MS: *m*/*z* (relative intensity): 229 (100), 275 (77.2), 202 (13), 167 (12.8), 135 (33.7); 121 (75.7, 109 (92.9), 95 (35.8), 65 (35.4), 41 (19.2).

FT-IR (film): 3296 (br), 3020, 2985, 1703, 1645, 1606, 1556, 1515, 1374, 1344, 1249, 1220, 1016, 993, 921, 832 cm^{-1}.

R_f: 0.46 (chloroform:acetone 1:8 v/v)

Preparation of 7 in the Presence of RAMEB. 550 mg (2 mmol) of **5**, 2.7 g (2 mmol) of RAMEB, and 10 mL of phosphate buffer (pH 7) were stirred at RT until a homogeneous solution was formed. After addition of 1.5 mg of HRP, 0.21 mL of 30% hydrogen peroxide solution was added slowly at intervals (0.1 mL/5 min). The mixture was stirred at RT for 15 h. The formed precipitate was collected by centrifugation. 332 mg of yellow-brown oligomer **7** was isolated after washing with acetone/water.

Preparation of 8. 243 mg of **7**, 8.2 mg (0.05 mmol) of AIBN, and some THF were introduced in an ampule. The solution was repeatedly vacuumized under refrigeration in fluid nitrogen for three times, so that it is thoroughly degassed. The ampule was then sealed under vacuum and heated at 65 °C for 2 days. The formed nonsticky mixture was powdered and washed with methanol. 204 mg of yellow hard powder was collected.

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