

SYNTHESIS OF POLYMERIC β -CYCLODEXTRIN SUPPORTED BY CROSSLINKED POLY(ACRYLAMIDE-*co*-VINYLAMINE) AND ITS CATALYSIS OF THE HYDROLYSIS OF *p*-NITROPHENYL ACETATE

Hong-jun Wang, Jian-biao Ma* and Bing-lin He

*The State Key Laboratory of Functional Polymer Materials for Adsorption and Separation,
Institute of Polymer Chemistry, Nankai University, Tianjin 300071, China*

Abstract Polymeric β -cyclodextrin (β -CD) supported by crosslinked poly(acrylamide-*co*-vinylamine) was synthesized as an artificial analog of hydrolytic enzyme and its catalysis of the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) was then investigated. The result showed that the polymer-supported β -CD could accelerate the hydrolytic reaction of *p*-NPA more quickly than β -CD itself and crosslinked poly(acrylamide-*co*-vinylamine) alone. The acceleration rate of the polymer-supported β -CD was about 10 times as fast as that of free β -CD in 0.01 mol/L phosphate buffer (pH 7.4) containing 32% DMSO at $37 \pm 0.1^\circ\text{C}$ when the molar amount of β -CD units in the polymer was equal to that of free β -CD in the experiments. The enhanced acceleration of the polymer-supported β -CD should be ascribed to the cooperative contribution of the inclusion effect of β -CD ring and the nucleophilic effect of amino groups on the polymeric support.

Keywords Polymer-supported catalysis, β -Cyclodextrin, Crosslinked poly(acrylamide-*co*-vinylamine), Hydrolysis of *p*-nitrophenyl acetate

INTRODUCTION

The catalytic activity of cyclodextrin was first reported by Cramer^[1] and has been found in many reactions^[2-7]. The catalysis can be ascribed to its ability to form binary complexes by inclusion of hydrophobic guest molecules within its hydrophobic cavity. This resembles enzymatic catalysis (hydrophobic cavity as binding site and hydroxyl or amino group as catalytic site). Thus, cyclodextrin and its small molecular derivatives could be designed as the enzyme-analog catalysts. Many attempts have been made to construct artificial enzymes capable of both inclusion and catalysis in bioorganic chemistry^[8-12]. In our present work, β -CD was bound onto crosslinked poly(acrylamide-*co*-vinylamine) to fabricate a polymeric enzyme-analog catalyst as shown in Scheme 1. Hydrolysis of *p*-nitrophenyl acetate as a model substrate was selected to assess the catalytic activities of the polymer-supported β -CD compared with free β -CD.

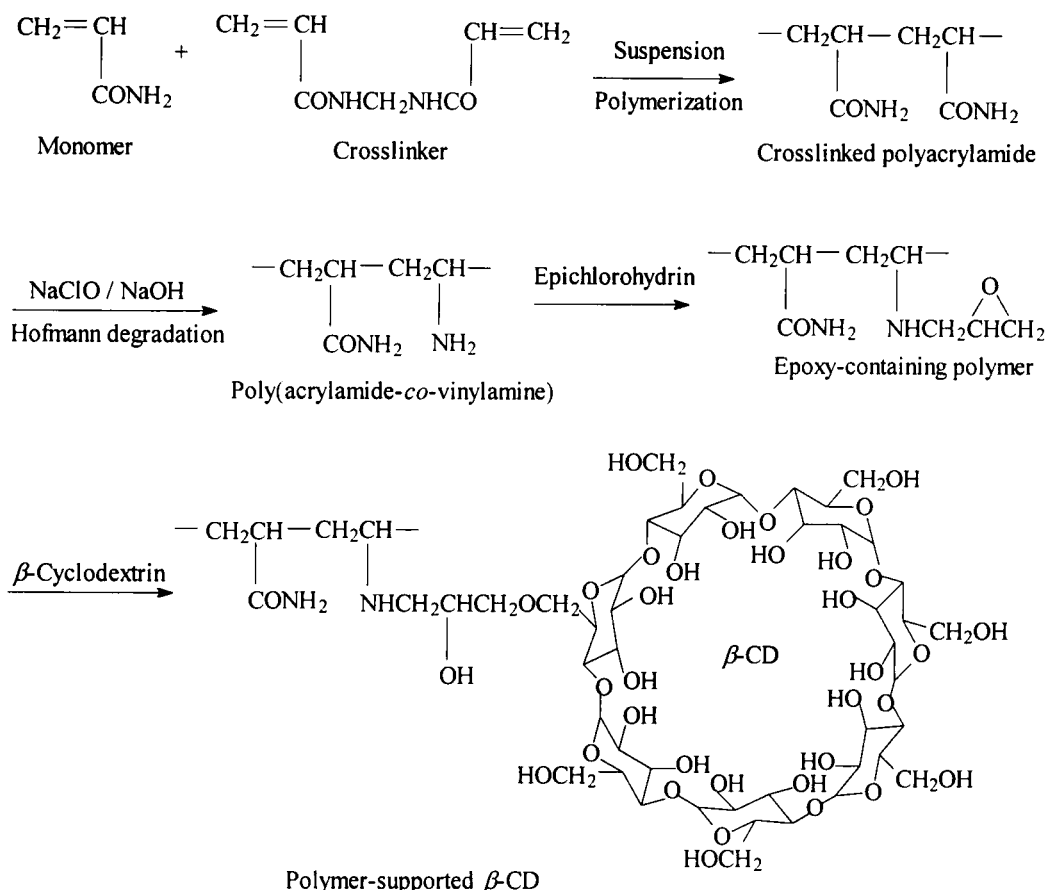
EXPERIMENTAL

Materials

β -Cyclodextrin (95%) was recrystallized from distilled water and dried under vacuum at 110°C for two days. Tetrahydrofuran (THF) was distilled after drying over sodium. Dimethyl sulfoxide (DMSO) was dried over potassium hydroxide and distilled before use. *p*-Nitrophenyl acetate (m.p. $81\sim 82^\circ\text{C}$) was prepared from *p*-nitrophenol and acetic chloride in dried THF and recrystallized three times from distilled water.

* Corresponding author: Jian-biao Ma (马建标), E-mail: sorp@sun.nankai.edu.cn

Received June 21, 1999



Scheme 1 The route for synthesis of the polymer-supported β -cyclodextrin

Synthesis of the Polymer-Supported β -CD

Crosslinked polyacrylamide beads with a particle size range of 40–60 mesh were prepared by the reversed-phase suspension polymerization of an aqueous solution of acrylamide as monomer and *N,N*-methylene-bisacrylamide as crosslinker^[13]. The beads underwent Hofmann degradation in ice-water bath to give crosslinked poly(acrylamide-co-vinylamine) beads^[14]. The content of amino groups in crosslinked poly(acrylamide-co-vinylamine) was determined as 8.04 mmol/g by acid-base titration.

Crosslinked poly(acrylamide-co-vinylamine) beads were well swollen in aqueous NaOH solution (0.55 mol/L) and then epichlorohydrin was added (1:1 mol/mol to amino groups in the polymer). The mixture was stirred at 40°C for 6 h to obtain the epoxy-containing polymer. β -CD was dissolved in an aqueous NaOH solution (0.55 mol/L) and the epoxy-containing polymer was added. The mixture was heated at 95°C for 9 h in an oil bath. After the product was washed successively with large amount of deionized water, acetone and finally in a Soxhlet apparatus with acetone for one day, it was dried at 40°C in vacuum. The β -CD units in the product were determined as 49.6 $\mu\text{mol/g}$ using the method described previously^[15].

Hydrolysis of *p*-NPA in the Presence of Catalyst

Working curve

Accurately weighed *p*-nitrophenol (0.07 g) was dissolved in 0.01 mol/L phosphate buffer (pH = 7.4) in a 100 mL volumetric flask. Then the solution was diluted with the phosphate buffer to different concentrations i.e. 5, 10, 20, 30, 35, 40, 50 $\mu\text{mol/L}$. Their absorbances were measured at 405 nm by UV spectrophotometry. The working curve of absorbance versus *p*-nitrophenol concentration was drawn.

The determination of hydrolysis of *p*-NPA

Phosphate buffer (0.01 mol/L, pH = 7.4, 15 mL), DMSO (7 mL) and a certain amount of a catalyst were mixed in a flask with a ground-glass stopper. 1 mL 0.04 mol/L *p*-NPA in the phosphate buffer was added to the mixture and timing started immediately. The flask was kept in a thermostat with shaking device at 37 °C. The spectrophotometric absorbance was measured at 405 nm on a 721 spectrophotometer. The concentration of *p*-nitrophenol in the system at different times was determined according to the working curve and the corresponding conversion percentage of *p*-NPA was calculated.

RESULTS AND DISCUSSION

Synthesis of the Polymer-Supported β -CD

The polymer-supported β -CD was synthesized from polyacrylamide as shown in Scheme 1. Crosslinked polyacrylamide in the form of polymeric beads was prepared by suspension polymerization and a part of its amide groups was changed into amino groups through Hofmann degradation as described previously^[14]. It was calculated from the content of amino group (8.04 mmol/g) that the crosslinked poly(acrylamide-*co*-vinylamine) contained 46.6% vinylamine units. Such an amino resin was activated by epichlorohydrin and then reacted with β -CD to afford the polymeric β -CD, containing 49.6 μ mol β -CD units /g. It was found that the number of β -CD units on the polymer was much less than the amino content in crosslinked poly(acrylamide-*co*-vinylamine). The first reason should be that the additional crosslinking bonds would be formed during the reaction of crosslinked poly(acrylamide-*co*-vinylamine) with epichlorohydrin resulting in a decrease of swelling ratio in water^[13]. The second one was that the β -CD has a high molecular weight so that it could not enter the inner part of the crosslinked polymeric beads to react with epoxy groups. In addition, more than one hydroxy groups in a β -CD molecule with twenty-one hydroxy groups might take part in the reaction. Thus it is easily understood why a low amount of β -CD was anchored onto the polymeric support.

The Hydrolysis of *p*-NPA Catalyzed by Free β -Cyclodextrin

The acceleration effect of β -CD at different concentration on the hydrolysis of *p*-NPA was investigated. The hydrolysis kinetics of *p*-NPA catalyzed by free β -CD under different conditions is demonstrated in Fig. 1. The results show that the hydrolysis rate of *p*-NPA increases as the concentration of β -CD is increased. It is known that cyclodextrin contains a hydrophobic cavity and hydroxy groups are located on its rim. *p*-NPA could insert its

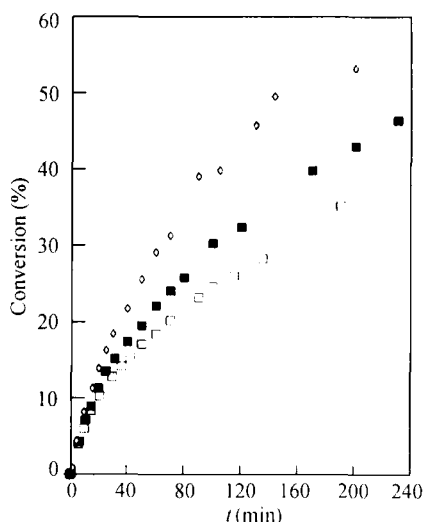
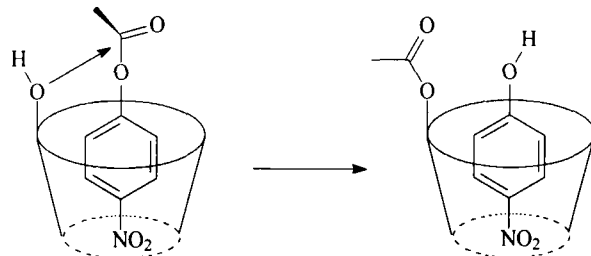


Fig. 1 The hydrolytic kinetics of *p*-NPA catalyzed by β -CD
a) no catalyst ($\square$), b) $[\beta\text{-CD}]:[p\text{-NPA}] = 0.26$ ($\blacksquare$),
c) $[\beta\text{-CD}]:[p\text{-NPA}] = 1.28$ ($\diamond$)

nitrophenyl group into the cavity of β -CD to give an inclusion complex. The hydroxy groups on the edge of the β -CD cavity might be regarded as the nucleophile. Obviously, the hydroxy groups of β -CD or the nearby water molecules would easily attack the ester bond of p -NPA over the cavity as shown in Scheme 2. Therefore, the hydrolysis of p -NPA was accelerated.



Scheme 2 The catalytic mechanism of β -CD for the hydrolysis of p -NPA

Catalysis of the Polymer-Supported β -CD for the Hydrolysis of p -NPA

The kinetics of p -NPA hydrolysis in the presence of the polymer-supported β -CD (1.56 mmol/L β -CD units) was examined and shown as the curve c in Fig. 2. The polymer-supported β -CD obviously possessed higher catalytic activity than the crosslinked poly(acrylamide-*co*-vinylamine) beads (the curve b in Fig. 2) and much higher than no catalyst (the curve a in the Fig. 2). It is known that a nucleophile^[16] can commonly facilitate the hydrolysis of p -NPA. The secondary amino groups on the polymeric carrier might act as the nucleophiles and would make the hydrolytic process faster. The high catalytic activity of the polymer-supported β -CD is due to a perfect combination of efficient inclusion of p -NPA in the β -CD cavity of the polymer-supported β -CD and an effective nucleophilic attack by a nearby amino group on the polymeric carrier, as demonstrated in Scheme 3. On the other hand, the higher catalytic activity of the polymeric β -CD than the free β -CD also indicated that the amino groups on the support are more catalytically effective than the hydroxyl group of the β -CD rim because of the greater nucleophilicity of an amino group compared to a hydroxyl group. The polymer-supported β -CD may be regarded as either a derivative of β -CD with a convergent nucleophile located above the β -CD cavity or a poly(acrylamide-*co*-vinylamine) derivative containing a specific binding site. When the molar amount of β -CD units in the polymeric catalyst was equal to that of free β -CD in the experiments, the acceleration rate of the

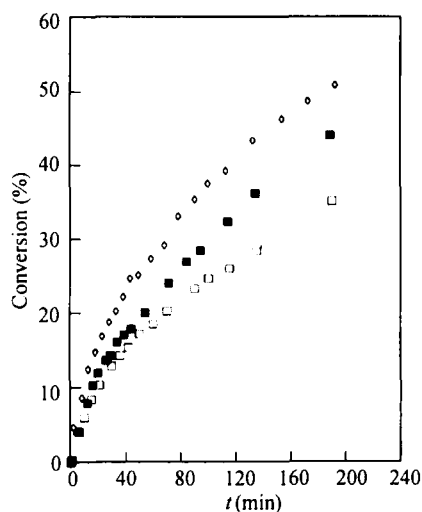
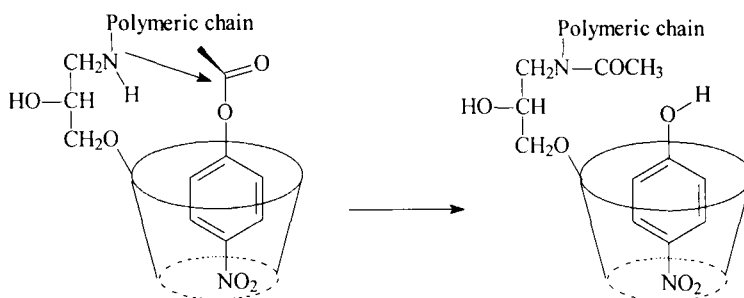


Fig. 2 The catalysis of the polymer-supported β -CD in the hydrolysis of p -NPA
a) no catalyst (□), b) crosslinked poly(acrylamide-*co*-vinylamine) (■),
c) the polymer-supported β -CD (◇)

polymer-supported β -CD was about 10 times as fast as that of free β -CD in 0.01 mol/L phosphate buffer (pH 7.4) containing 32% DMSO at $37 \pm 0.1^\circ\text{C}$.



Scheme 3 The catalytic mechanism of the polymer-supported β -CD for the hydrolysis of *p*-NPA

The catalysis by crosslinked poly(acrylamide-co-vinylamine) alone and free β -CD was compared. When they were used in a comparison experiment with equal molar amounts of free β -CD and amino units in the polymer, their catalytic activities were almost same as shown in Fig. 3. It was drawn from a rough estimation that the catalytic effect of the polymer-supported β -CD was almost the sum of those of crosslinked poly(acrylamide-co-vinylamine) and free β -CD. However, after β -CD was linked onto the polymeric beads, only those secondary amino groups on the surface of the polymeric beads, less than the total amino groups, might exert their catalytic effect because the hydrophobic *p*-NPA could not get into the inner portion of the highly crosslinked polymer. Therefore, the combination of inclusion in a β -CD cavity and a nucleophilic attack of a nearby amino group on the polymer-supported β -CD should bring about a greater acceleration of *p*-NPA hydrolysis than the simple addition of the catalytic effects of amino groups and β -CD units.

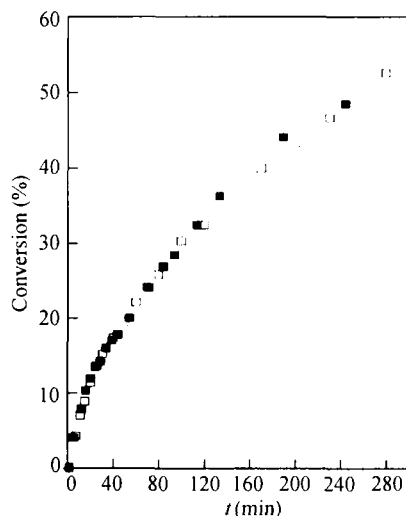


Fig. 3 The comparison in catalysis of crosslinked poly(acrylamide-co-vinylamine) and free β -CD

a) crosslinked poly(acrylamide-co-vinylamine) (■), b) free β -CD (□)

The Final Conversion Percentage of the *p*-NPA Hydrolysis in Different Systems

Generally speaking, a catalyst can only accelerate the rate of a reaction, but it cannot change the reaction equilibrium. That is true for the hydrolysis of *p*-NPA catalyzed by the polymer-supported β -CD or free β -CD or its amino support alone. The results in Table 1 show that the equilibrium conversion keeps almost constant under different conditions, suggesting that the polymer-supported β -CD played a catalytic role only during the hydrolytic reaction of *p*-NPA and does not affect its thermodynamic equilibrium, just like a common catalyst.

Table 1. The equilibrium conversion of the hydrolysis of *p*-NPA in different systems

Reaction system	I	II	III	IV	V
Catalyst	No catalyst	β -CD ([β -CD]<[S])*	β -CD ([β -CD]>[S])*	Polymeric β -CD	Poly(acrylamide- co-vinylamine)
Conversion (%)	96.7	96.5	96.6	96.7	96.7

*[S] is the initial concentration of the substrate *p*-NPA

CONCLUSION

The polymer-supported β -CD bound onto crosslinked poly(acrylamide-*co*-vinylamine) was proved to be an effective artificial enzyme catalyst, which could accelerate the hydrolysis of *p*-NPA as a model substrate more efficiently than crosslinked poly(acrylamide-*co*-vinylamine) or free β -CD at equal molar concentrations. Regarding its catalytic mechanism it is suggested that the *p*-nitrophenyl group of *p*-NPA enters into the cavity of β -CD and an amino group on the polymeric support near the β -CD unit attacks the bare ester group so as to facilitate the hydrolysis of *p*-NPA.

ACKNOWLEDGEMENT We acknowledge the National Natural Science Foundation of China for its financial support.

REFERENCES

- 1 Cramer, F., Chem. Ber., 1953, 86: 1576
- 2 Cramer, F. and Dietsche, W., Chem. Ind. (London), 1958, (28): 892
- 3 Hennrich, N. and Cramer, F., J. Am. Chem. Soc., 1965, 87: 1121
- 4 Cramer, F., Angew. Chem., 1961, 73: 49
- 5 Brass, H.J. and Bender, M.L., J. Am. Chem. Soc., 1973, 95: 5391
- 6 Congdon, W.I. and Bender, M.L., Bioorg. Chem., 1971, 1: 424
- 7 Giffiths, D.W. and Bender, M.L., J. Am. Chem. Soc., 1973, 95: 1679
- 8 Akiyama, M., Ohmachi, T. and Ihjima, M., J. Chem. Soc., Perkin Trans. 2, 1982, (12): 1511
- 9 Cramer, F. and Georg, M., Chem. Ber., 1970, 103: 2138
- 10 Breslow, R. and Campbell, P., Bioorg. Chem., 1971, 1: 140
- 11 Breslow, R., Kohn, H. and Siegel, B., Tetrahedron Lett., 1976, (20): 1645
- 12 Breslow, R. and Chung, S., J. Am. Chem. Soc., 1990, 112: 9659
- 13 Wang, H.J., Ma, J.B., Zhang, Y.H. and He, B.L., React. Funct. Polym., 1997, 32: 1
- 14 Yuan, Z., Shen, L.H., Yang, D.F. and He, B.L., Chem. J. Chinese Univ. (Chinese Ed.), 1997, 18: 154
- 15 He, B.L., Zong, F. and Ma J.B., Scientia Sinica (Part B, Chinese Ed.), 1993, 23: 225
- 16 Yamashita, K., Kimura, Y., Saba, H. and Tsuda K., J. Polym. Sci., Part A, 1991, 29: 777