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Vijayendra Kumar[ab]; Virinder S. Parmar[ab]; Lynne A. Samuelson[c]; Jayant Kumar[a]; Ashok L. Cholli[a]
[a] Center for Advanced Materials, Departments of Chemistry and Physics, University of Massachusetts—Lowell, Lowell, MA, U.S.A. [b] Department of Chemistry, University of Delhi, Delhi, India [c] U.S. Army Soldier and Biological Chemical Command, Development and Engineering Center, Natick Soldier Center, Natick, MA, U.S.A.

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ENZYME MEDIATED OXIDATIVE POLYMERIZATION OF 4-HYDROXYBENZYL ALCOHOL FOR OPTICAL APPLICATIONS

Vijayendra Kumar,[1,2] Virinder S. Parmar,[1,2] Lynne A. Samuelson,[3] Jayant Kumar,[1] and Ashok L. Cholli[1,*]

[1]Center for Advanced Materials, Departments of Chemistry and Physics, University of Massachusetts—Lowell, Lowell, MA 01854
[2]Department of Chemistry, University of Delhi, Delhi 110 007, India
[3]Natick Soldier Center, U.S. Army Soldier and Biological Chemical Command, Development and Engineering Center, Natick, MA 01760

ABSTRACT

Poly(4-hydroxybenzyl alcohol) was synthesized enzymatically starting with 4-hydroxybenzyl alcohol using hydrogen peroxide as the oxidizing agent in methanol and 0.01 M phosphate buffer (pH 7.0) mixture (1:1, v/v) and at room temperature (25°C). Polymerization reaction was catalyzed by horseradish peroxidase (HRP). The polymer formed was characterized by UV-Vis, FT-IR, ^{1}H and ^{13}C-NMR spectroscopic studies, which revealed that HRP catalyzed polymer is composed of a mixture of phenylene and oxyphenylene units and alcoholic hydroxyl groups are not participating in the polymerization. The resulting poly(4-hydroxybenzyl alcohol) contains pendant primary hydroxyl groups which provide an opportunity to further functionalize the polymer. The post-functionali-

*Corresponding author. E-mail: Ashok_Cholli@uml.edu

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zation of enzymatically synthesized polymers with photoresponsive chromophores provides new molecularly engineered materials that are useful in photonics and sensor applications.

Key Words: Enzymatic polymerization; Horseradish peroxidase; Poly (4-hydroxybenzyl alcohol); Characterization; ^{1}H-NMR; ^{13}C-NMR; IR; UV-Vis spectroscopy

INTRODUCTION

Typical conventional polymers using phenolic compounds as monomers are phenol formaldehyde-resins.[1] These polymers are widely used in various fields, such as wood composites, fiber bonding, laminates, foundry resins, abrasives, friction and molding materials, coatings and adhesives, and flame retardants.[2] There is a serious concern about the continued use of phenol-formaldehyde resins due to various toxic effects of formaldehyde and harsh reaction conditions during polymerization.[3,4] After the report of Klibanov et al. on the peroxidase-catalyzed polymerization of p-phenylphenol in an aqueous organic solvent to produce a new class of polyphenols,[5] there has been much interest in polymerizations catalyzed by enzymes (''enzymatic polymerization'') as a new methodology of polymer synthesis[6] as it offers numerous advantages over the conventional methods. Enzymatic polymerization reactions can offer mild and environment-friendly reaction conditions, high degree of specificity, control over the kinetics of reaction, minimal biproduct formation, efficient procedure for polymerization as well as polymer isolation, handle on control of structure and solubility of polymer and high yields of products.[7] Recently, enzymatic synthesis of polyaromatics has been extensively developed.[8–13] Klibanov et al.[5] initially used the enzyme, horseradish peroxidase (HRP) for enzymatic synthesis of polyphenols. Since then, a variety of phenols and aromatic amines were oxidatively polymerized by horseradish peroxidase under a wide range of mild reaction conditions including aqueous and non–aqueous media, micelles, reverse micelles and polymerization at air-water interface to produce novel polymeric materials consisting of a mixture of phenylene and oxyphenylene units.[14–19] The synthesized polyphenols and polyanilines show interesting optical and electrical properties with potential applications in the fabrication of optical and electronic devices.[20]

Peroxidase-catalyzed polymerization reaction is an oxidation of a donor to an oxidized donor by the action of hydrogen peroxide, resulting in the elimination of water molecules. Horseradish peroxidase (HRP) is a single chain β-type hemoprotein that catalyzes the decomposition of hydrogen peroxide at the expense of aromatic proton donors, it has a Fe-containing porphyrin-type structure and is well known to catalyze a number of phenol and aniline derivatives using hydrogen peroxide as oxidant.[21] The catalytic

cycle of peroxidase involves a two-electron oxidation step and two one electron reduction steps, resulting in the formation of phenolic radicals.[22] These free radicals then undergo coupling to form polymeric chains. Generation of radicals is enzyme-dependent, whereas radical-radical coupling and transfer are controlled by phenoxy radical and solvent chemistry.[23] The polymer obtained by peroxidase-catalysis, usually possess complicated structure, but the polymer structure was mainly estimated to be a mixture of phenylene and oxyphenylene units.[5] In the present work, a novel polymeric material was enzymatically synthesized starting with 4-hy droxybenzyl alcohol. The polymer formed by the peroxidase-catalyzed oxidative polymerization of 4-hydroxybenzyl alcohol contains pendant hydroxyl groups, thus the new polymer offers a unique system where repeat units have primary hydroxyl groups that are available for post-coupling reactions with optically active chromophores. The tailorability of this new polymer has potential for the development of functionalized polymers for optical and sensor applications.

EXPERIMENTAL

Materials

Horseradish peroxidase (HRP) (200 unit/mg) was purchased from Sigma Chemical Co., St. Louis, MO. The monomer 4-hydroxybenzyl alcohol (purity 99%), sodium dihydrogenphosphate and hydrogen peroxide (50% by weight) were purchased from Aldrich Chemical Co. Inc., Milwaukee, WI and were used as received. All other chemicals used were of reagent grade or better and used as received.

Synthesis of Poly(4-Hydroxybenzyl Alcohol)

4-hydroxybenzyl alcohol (0.124 g, 1.0 mmol) was dissolved in 50% methanol and 50% 0.01 M sodium phosphate buffer (pH 7.0) mixture (10 mL) at room temperature (25°C). HRP (1.0 mg) was added to this solution. The polymerization was initiated by incremental addition of a stoichiometric amount of hydrogen peroxide (5% aq. solution, 1.15 mL, 1.0 mmol). Dropwise addition of hydrogen peroxide to the mixture was continued after every 15 min over a period of 3 h. After complete addition of H_2O_2, the reaction was allowed to continue for 24 h. The greenish-yellow precipitate formed during the reaction was collected by centrifugation. The precipitate was washed thoroughly with aqueous methanol (1:1, v/v) to remove the enzyme, phosphate salts and unreacted monomer, followed by drying in vacuum for 24 h to give the polymer.

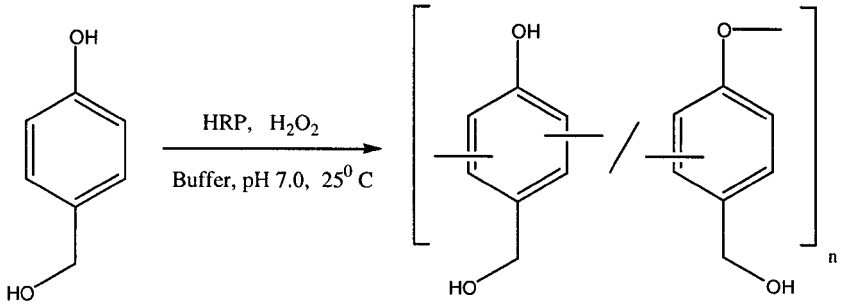
Characterization

Fourier-transform infrared spectra (FT-IR) for both the monomer and the polymer were obtained by using a Perkin-Elmer 1720 FT-IR spectrometer. Pellets were prepared by mixing KBr with samples for FT-IR study. UV-Vis absorption spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of monomer and polymer in dimethyl sulfoxide $-d_6$ (DMSO-d_6) were obtained on a Bruker ARX-500 NMR spectrometer. The thermal properties of the polymer were measured with a DuPont thermal analyzer, TGA 2950 (TA Instruments Inc.).

RESULTS AND DISCUSSION

The HRP-catalyzed oxidative polymerization of 4-hydroxybenzyl alcohol was carried out in an equivolume mixture of methanol and 0.01 M phosphate buffer (pH 7) at 25°C as schematically depicted in Scheme 1.

These polymerizations were also carried out by using the equivolume amounts of acetone and 0.01 M phosphate buffer (pH 7.0), and 1,4-dioxane and 0.01 M phosphate buffer (pH 7.0). The polymers formed using acetone and 1,4 dioxane solvent systems were not soluble in any organic solvent. The polymerization was initiated by incremental addition of a stoichiometric amount of hydrogen peroxide (oxidizing agent) to the mixture to avoid the inhibition of HRP.[5] During the polymerization of 4-hydroxybenzyl alcohol in methanol, the reaction mixture turned turbid with the addition of hydrogen peroxide, followed by the formation of a greenish precipitate. The color of the precipitate gradually became greenish yellow. After 24 h, powdery material was collected by centrifugation and washed thoroughly with aqueous methanol (1:1, v/v) to remove the enzyme, phosphate salts and



Scheme 1. HRP-mediated polymerization of 4-hydroxybenzyl alcohol.

unreacted monomer. The solid material obtained was dried in vacuum for 24 h The resulting polymer was soluble in *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) but insoluble in water and other common organic solvents.

The polymer structure was analyzed by FT-IR, ^{1}H and ^{13}C-NMR spectroscopic methods. Fourier transform infrared (FT-IR) spectra of monomer and polymer are shown in Fig. 1. Three important absorptions were observed in the spectra of the polymer. The first is broad peak from 3000 to 3700 cm^{-1} which shows nearly 90 cm^{-1} lower frequency shift with respect to monomer and is assigned to OH stretch and hydrogen bond formation in the polymer. The second absorption of interest is a broad peak centered at 1650 cm^{-1}, assigned to the conjugation of double bonds in the main chain. Third important absorption peak in the region 1150–1300 cm^{-1} is assigned to the asymmetric vibrations of the C-O-C linkages and to the C-OH vibrations of the polymer.[24] These data suggest that enzymatically synthesized polymer is composed of a mixture of phenylene and oxyphenylene units.

The ^{1}H-NMR spectra of the 4-hydroxybenzyl alcohol and poly(4-hydroxybenzyl alcohol) are shown in Fig. 2, the ^{1}H-NMR spectrum of the

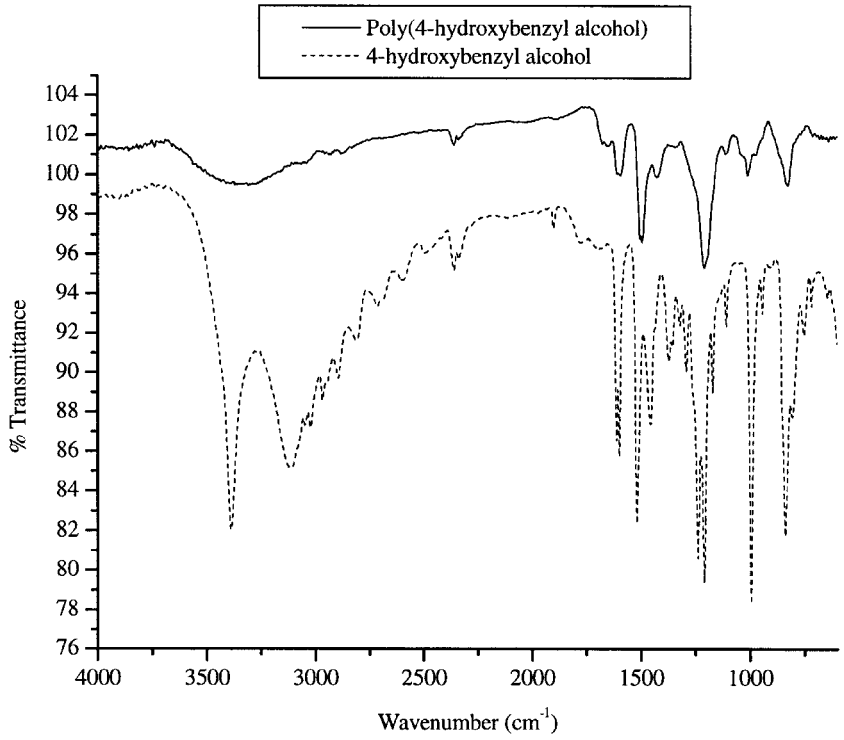


Figure 1. FT-IR spectra of 4-hydroxybenzyl alcohol and poly(4-hydroxybenzyl alcohol).

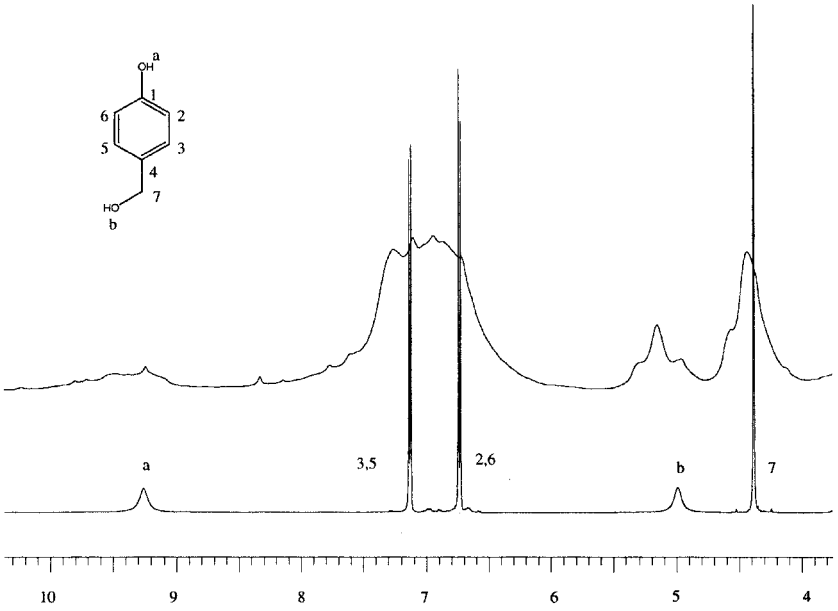


Figure 2. ^{1}H-NMR spectra of 4-hydroxybenzyl alcohol [bottom] and poly(4-hydroxybenzyl alcohol) [top].

monomer shows sharp peaks and the individual proton assignment for these peaks are given. The ^{1}H-NMR spectrum of the polymer showed broad peaks compared to the monomer, centered at about 4.4, 5.2, 6.9, and 9.2 ppm, the broadening of the peaks in the spectrum is attributed to the polymerization. In the ^{1}H-NMR spectrum of monomer, the integral ratio of the aromatic hydroxyl proton "a" in Fig. 2 to aliphatic hydroxyl proton "b" is 1:1, whereas, it is 1:1.7 for the polymer indicating that phenolic hydroxyl groups are participating in the coupling reaction, thus inferring the formation of C-O-C couplings in the polymer. In the ^{1}H-NMR spectrum of the polymer, the integral ratio of alcoholic hydroxyl to methylene remains same as in the monomer, thus indicating the alcoholic hydroxyl groups are not participating in the polymerization reaction. This relative decrease of integration of aromatic hydroxyl in the polymer as compared to monomer was also obtained in the case of 3-hydroxybenzyl alcohol.[25] The detailed analysis of ^{1}H-NMR spectral data suggests that there are ca. 40% C-O-C type coupling, while the remaining 60% coupling are in the form of C-C type in the polymer structure.

The ^{13}C-NMR spectrum of 4-hydroxybenzyl alcohol and its polymer are shown in Fig. 3. The ^{13}C-NMR spectrum of the monomer shows five sharp peaks and their assignments are shown in Fig. 3. The ^{13}C-NMR spectrum of the polymer showed broad signals and new resonances at 153.9 ppm, 138.2 ppm, 130.3 ppm, and 119.4 ppm in the aromatic region. The

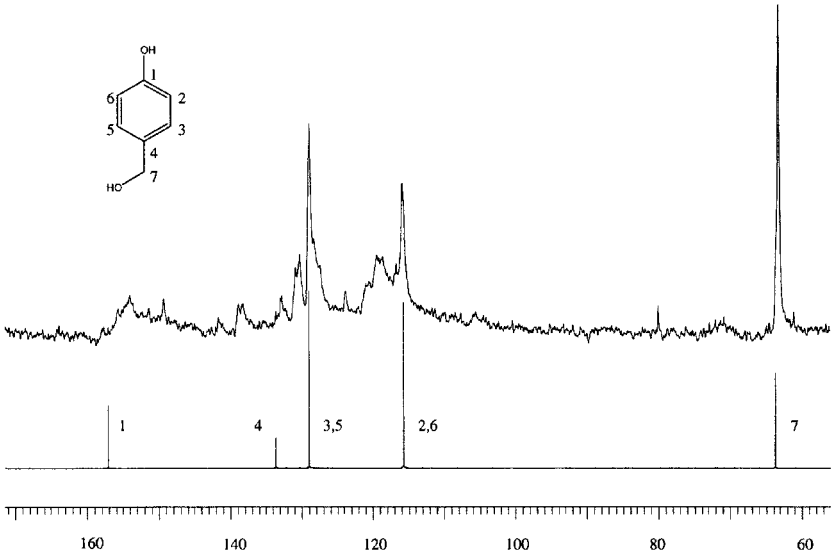


Figure 3. ^{13}C-NMR spectra of 4-hydroxybenzyl alcohol [bottom] and poly(4-hydroxybenzyl alcohol) [top].

peaks at 153.9 ppm and 138.2 ppm indicate the presence of C-O-C couplings in the polymer. Analysis of the methylene region of the ^{13}C NMR spectrum of the polymer showed single broad peak at 63.3 ppm whereas in case of poly(3-hydroxybenzyl alcohol), that region showed multiple resonances as a result of coupling taking place at the *ortho* and *para* positions to the phenolic hydroxyl group.[25] These data suggest that the in case of poly(4-hydroxybenzyl alcohol), polymerization is taking place at *ortho* positions of aromatic hydroxyl group whether it is in the form of C-C coupling or C-O-C coupling.

Figure 4 shows the UV-vis spectrum of 4-hydroxybenzyl alcohol and poly(4-hydroxybenzyl alcohol). Comparison of these spectra shows a significant absorption change that occurs as a result of polymerization. In the UV-vis spectrum of polymer, a large broad absorption tail is observed in the region from 325 to 800 nm and is attributed to the conjugation effect in polymer. A similar broadening of absorption has been observed for other enzymatically synthesized polyphenol systems.[16,24]

Thermogravimetric profiles of 4-hydroxybenzyl alcohol and poly(4-hydroxybenzyl alcohol) are shown in Fig. 5. Thermogravimetric analysis (TGA) were performed under nitrogen and a heating rate of 10°C/min was used. There is ca. 50% mass loss up to 400°C for monomer, whereas the polymer showed less than 15% mass loss up to 400°C, suggesting good thermal stability of the polymer.

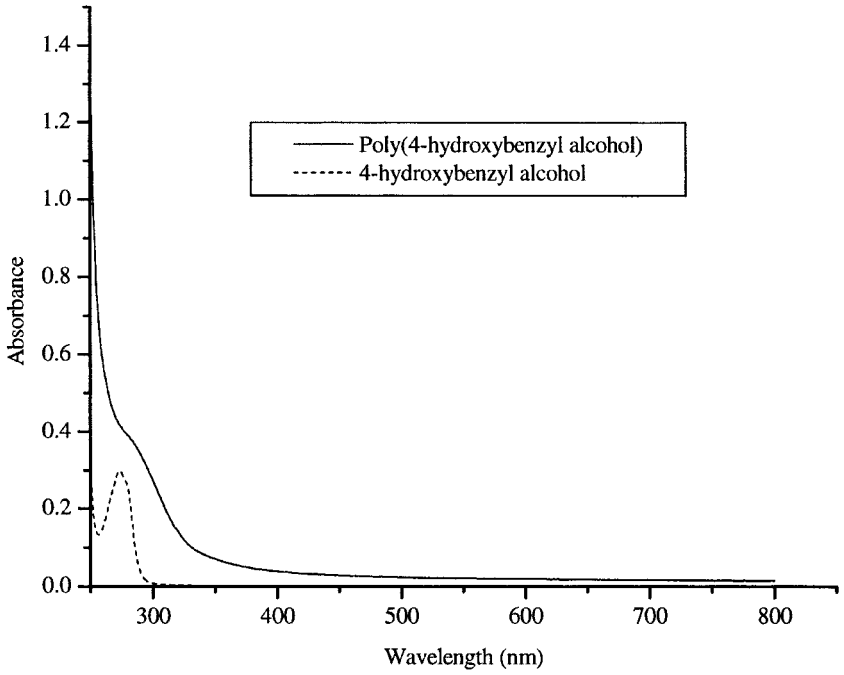


Figure 4. UV-Vis spectra of 4-hydroxybenzyl alcohol and poly(4-hydroxybenzyl alcohol).

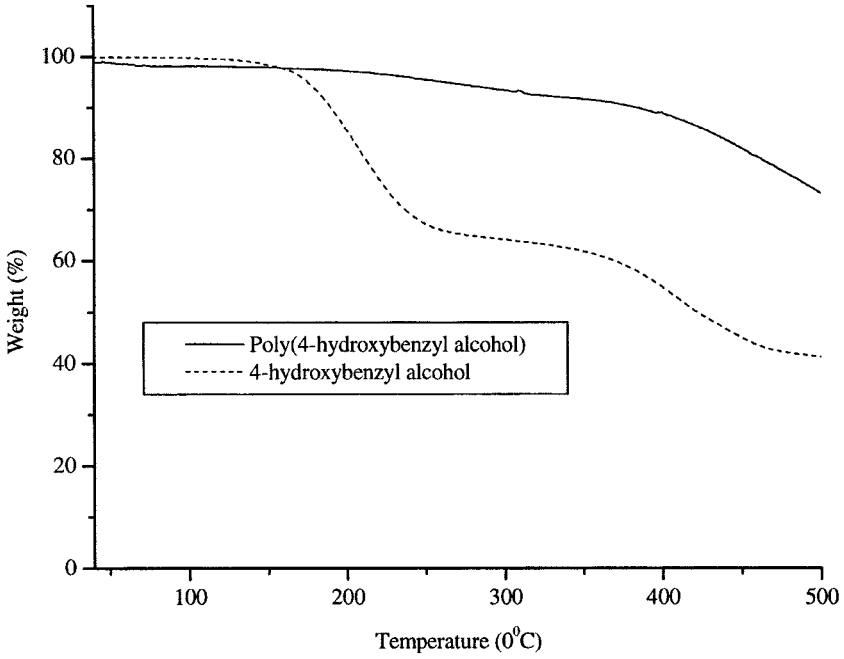


Figure 5. TGA profile of 4-hydroxybenzyl alcohol and poly(4-hydroxybenzyl alcohol).

The synthesized polymer contains pendant primary hydroxyl groups on every repeat unit, which may be difficult to synthesize by chemical means. These pendant groups provide an opportunity to further tailor the polymer for photonic applications by post-functionalizing the enzymatically prepared polymer with photoresponsive chromophores for writing surface-relief gratings and for preparing polymer membranes to sensor devices based on fluoroscence quenching mechanism. The work for post-coupling with azo-functionalized chromophores to poly(4-hydroxybenzyl alcohol) and subsequently, writing surface-relief gratings is under progress.

CONCLUSION

A new functionalized polyphenolic system has been prepared by HRP-mediated free radical polymerization of 4-hydroxybenzyl alcohol. This approach is simple, biochemically mild and requires minimal separation and purification. Detailed spectroscopic studies revealed that the coupling reaction occurs at *ortho* positions to the phenolic hydroxyl group of 4-hydroxybenzyl alcohol and the polymer structure was estimated to contain a mixture of phenylene and oxyphenylene units. The detailed analysis of ^{1}H NMR spectral data suggests that there are about 40% C-O-C type coupling, while the remaining 60% coupling are in the form of C-C type in the polymer structure. The thermogravimetric analysis shows that poly(4-hydroxybenzyl alcohol) has relatively high thermal stability.

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