

Characterization of polyaniline synthesized by enzyme-catalyzed reactions in organic solvents†

Joseph A Akkara*, P Salapu & David L Kaplan

Biotechnology Division, U S Army Natick Research, Development and Engineering Centre,
Natick, MA 01760-5020

Earlier studies have shown that properties of chemically synthesized polyaniline could be modified by the type of synthesis—electrochemical, chemical or potential cycling. A new enzymatic approach for the synthesis of polyaniline by the polymerization of aniline with hydrogen peroxide catalyzed by horseradish peroxidase in organic solvents with different amounts of water is reported. The polymer synthesized has been isolated and characterized for thermal properties, functional groups and structure. Thermal analyses indicate that the polyaniline synthesized has relatively good thermal stability with about 30 to 50% residue remaining after thermal treatment up to 600°C in an atmosphere of nitrogen. FTIR, ¹³C-NMR and ¹H-NMR spectra have indicated at least two possible structurally different polyaniline products, one consisting of alternating benzoid-quinoid structure and the other *ortho*- and *para*-substituted carbon-carbon and carbon-nitrogen bond structures.

Recent interest in polyaniline derives from its environmental stability, its electronic, optical and electrochemical properties, and its potential applications in electronic and electro-optic materials. Chemical synthesis of polyaniline was first carried out in 1840 and its primary use was as dyes¹. Recent studies on the synthesis of polyaniline have greatly contributed to the understanding of conditions and mechanisms involved in the polymerization reactions, and the properties of the polymer formed. The properties of polyaniline vary with the type of synthesis, namely electrochemical, chemical, or potential cycling methods²⁻⁵. In addition, recent studies have shown that the optical properties of polyaniline synthesized could be controlled by the substituents on the aromatic ring⁶.

A new biocatalytic approach for the synthesis of polyaniline is reported here. This polymerization is catalyzed by an enzyme-coupled H₂O₂-dependent oxidative polymerization of aniline in organic solvents containing different amounts of water. The physical and chemical properties of polyaniline synthesized by this method were studied to determine the differences, if any, between this polyaniline and those chemically synthesized.

Enzyme-catalyzed reactions carried out in organic solvents can offer many advantages when compared with reactions in aqueous systems, including improved solubility of reactants and products, in-

creased enzyme stability, and simplified recovery of enzyme and product^{7,8}. We have recently reported the horseradish peroxidase catalyzed reaction in dioxane for the synthesis of polymers from phenols and aromatic amines^{9,10}. We have also shown that molecular weight and other properties of the polymer products could be controlled by the reaction conditions used.

Materials and Methods

Horseradish peroxidase (EC 1.11.1.7, Type II, 150-200 units/mg solid), hydrogen peroxide (30%), and buffers were purchased from Sigma Chemical Company (St. Louis, MO). Aniline and other chemicals were obtained from Aldrich Chemical Company (Milwaukee, WI). Solvents used were High Performance Liquid Chromatography (HPLC) grade and were purchased from Caledon Laboratories (Ontario, Canada).

Enzymatic synthesis

The enzymatic synthesis of polyanilines was carried out with horseradish peroxidase-catalyzed H₂O₂-dependent reactions in solvents, *N,N*-dimethyl formamide, dioxane, tetrahydrofuran, toluene, and dichloromethane with different amounts of HEPES buffer (pH 7.5) (*N*-[2-hydroxyethyl]piperazine *N'*-[ethane sulfonic acid]) at room temperature. Aniline was dissolved in the solvent, and the enzyme dissolved in the buffer was added to the solvent solution in small increments. The polymerization reaction was initiated by adding

† Presented at the IUPAC-NOST International Symposium on "Enzymes in Organic Synthesis" held in New Delhi during January 6-9, 1992.

the H_2O_2 solution to the above reaction mixture. The synthesis was terminated after 18 hr of incubation and the polymer isolated by centrifugation. The polymer was then washed with water followed by the solvent used in the reaction to remove residual buffer, horseradish peroxidase, unreacted monomers and low molecular weight oligomers. Other details for the synthetic approach were as described earlier^{9,10}.

Chemical synthesis

The aniline solution in 1 M HCl was oxidized by ammonium persulfate solution at 4°C. The molar ratio of the oxidant to monomer was 0.25 to 1. Other details of the chemical polymerization of aniline were described earlier¹¹.

Instrumentation

Melting point was determined by a bench top capillary point apparatus (Thomas Hoover, Arthur H. Thomas Co., Philadelphia, PA). Thermal properties were determined by a Thermal Gravimetric Analyzer (TGA) (Model 951 Du Pont, Wilmington, DE) and Differential Scanning Calorimetry (DSC) (Model 910, Du Pont) by heating the polymer up to 600°C (TGA) and 250°C (DSC) in a stream of nitrogen gas. For thermal analyses (TGA and DSC) the flow rate of nitrogen gas was set at 70 ml/min, the sample size was 2 to 10 mg, and the temperature scan rate was 5°C or 10°C/min. The data were analyzed by the Thermal Analyzer (Model 1090, Du Pont) with TGA Analysis V1.0 program (Du Pont) and Interactive DSC V2.0 program (Du Pont). Fourier Transform Infrared (FTIR) spectroscopy was performed on a Nicolet (Model 20SXB, Madison, WI) and spectroscopic properties of the polymers were evaluated using KBr pellets at 1% (w/w) polymer concentration. ¹³C solid-state NMR of polymers was carried out with cross-polarization magic-angle sample spinning (CP/MASS), at a spin speed of 3.8-4.3 kHz (Spectral Data Service, Inc., Champaign, IL). Chemical shifts were calibrated through the external standard of tetramethylsilane. A deconvolution program was used for the partition and identification of the ¹³C-NMR peaks. Proton NMR of a polyaniline solution in dimethyl-*d*₆-sulfoxide was determined at 360 MHz (Nicolet, Model # 360, Madison, WI). Amino protons in the polymer were confirmed by D₂O exchange studies.

Results and Discussion

Polyaniline was synthesized by enzyme-catalyzed reactions in an organic solvent (dimethylformamide, tetrahydrofuran, dioxane, toluene and dichloromethane) containing 5 to 60% buffer. The enzymatic

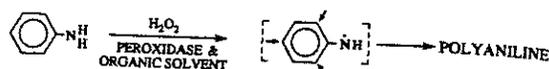


Fig. 1—Schematic diagram of the polyaniline synthesis by the enzyme-catalyzed reaction

reaction in dimethylformamide, dioxane and tetrahydrofuran was monophasic; however, the reaction in toluene and dichloromethane was biphasic. The schematic of the reaction is given in Fig. 1.

Table 1 lists organic solvents (with different amounts of the buffer) used for the synthesis of polyaniline by enzyme catalyzed reaction and the yield of polymers. The polymer yield was determined after the reaction products were separated by centrifugation and washing as described above. Because of the washing procedure adopted, low molecular weight polymers were removed from the polymer collected and this may explain the low yield observed with some of the solvents listed in Table 1. The polymer yield was very low when the enzymatic reactions were carried out in toluene and dichloromethane with different amounts of buffer.

Thermal analyses of the polymers synthesized by enzyme-catalyzed reaction indicated that the melting or softening point of the polymer varied from 260 to 270°C (see Table 2). TGA analyses (in nitrogen gas) of the polyanilines prepared under dif-

Table 1—Solvent systems used for the enzymatic synthesis of polyaniline and the polymer yield

Solvent	Solvent/buffer ratio	Polymer yield (%)
<i>N,N</i> -dimethylformamide	40/60	6.1
	50/50	8.4
	60/40	7.4
	70/30	4.9
	80/20	†
	90/10	†
1,4-Dioxane	70/30	3.7
	80/20	1.9
	90/10	1.7
	95/5	†
Tetrahydrofuran	50/50	1.9
	60/40	†
	70/30	1.4
	80/20	2.2
	90/10	†
Toluene*		
Dichloromethane*†		

*Evaluated at 70/30, 80/20 & 90/10 solvent/buffer ratios.

†No precipitate separated or poor yield.

Table 2—Thermal analysis of the polyaniline synthesized by the enzyme catalyzed reaction

Solvent system	m.p. (°C)	TGA (°C)*	DSC (°C)†	
<i>N,N</i> -dimethylformamide/ HEPES buffer	40/60	260-270	334	90.5‡
				208.6§
				80.0‡
				161.3§
				231.4§
60/40	260-270	384	84.7‡	
			175.0§	
			202.0§	
70/30	260-270	340	107.2‡	
			202.0§	
1,4-Dioxane/ HEPES buffer	70/30	260-270	340	107.2‡
				200.0§
				95.8‡
80/20	260-270	335	190.0§	
			95.8‡	
90/10	260-270	343	190.0§	
			95.8‡	
			190.0§	
Tetrahydrofuran/ HEPES buffer	70/30	286	190.0§	
			317	

*The temperature midpoint of the major weight loss.

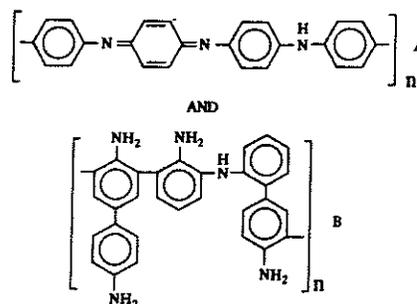
†The temperature given is from the first heat data.

‡Endotherm

§Exotherm

ferent reaction conditions showed only one major weight loss (up to 50%) in the temperature range of 286 to 380°C and up to 50% residue remained after heating to 600°C. However, TGA of chemically synthesized polyaniline indicated three smaller stages of weight loss of 10.2%, 11.3% and 11.5% at 70°C, 252°C and 511°C, respectively, with a total loss of 33% when heated to 1000°C. DSC analyses of different enzymatically synthesized polyaniline preparations indicated that polymers have broad endotherm around 80-180°C followed by a broad exotherm at around 160-230°C. The endotherm-exotherm combination could be due to gelling of the polymer followed by cross-linking. The DSC analyses of chemically synthesized polyaniline indicated a broad endotherm at 108°C followed by a partially overlapping exotherm from 175°C when the sample was heated up to 300°C. Thermal stability and heat flow of chemically synthesized polyaniline were similar to those reported earlier¹³.

The FTIR spectrum of the enzymatically synthe-

**Fig. 2**—Possible structures of polyaniline synthesized by enzyme-catalyzed reactions in organic solvents

ized polyaniline (in dimethyl formamide/buffer in the ratio 60:40, see Table 1) indicated both primary and secondary amines (a broad peak around 3286 cm^{-1} for N-H stretching vibrations and a peak at 1300 cm^{-1} for C-N stretching vibrations). In addition, quinoidal structures (see Fig. 2A) are indicated by peaks at 1560 cm^{-1} and 1591 cm^{-1} for C=N stretches. Multiple substitutions on the aromatic ring are indicated by C-H out-of-plane bending bands around 800 cm^{-1} . Chemically synthesized polyaniline had no peaks for primary and secondary amines and had a C=N stretch vibration (for quinoid structures; see Figure 2A) peak at 1586 cm^{-1} . The FTIR spectrum of the chemically synthesized polyaniline was similar to those reported in literature^{6,12,13}.

¹³C solid-state NMR of polyaniline synthesized by enzyme-catalyzed reaction (in dimethyl formamide/buffer in the ratio 70:30, see Table 1) has shown the presence of four major resonances centered at 121 (with shoulders around 115 and 125 ppm), 128.7, 138.7 and 150 ppm and a minor one at 155.2 ppm. The resonances at 125, 138.7 and 155.2 ppm indicated the presence of many localized double bonds in an alternating benzoid-quinoid structure linked with an imine at *para*-positions as shown in Fig. 2A. Earlier NMR studies with chemically synthesized polyanilines have indicated the presence of a similar alternating benzoid-quinoid structure¹⁴. In addition, the presence of *ortho*- and *para*-substituted, carbon-carbon and carbon-nitrogen bonds was also indicated in the biocatalysis-derived polyaniline by the presence of resonances at 121, 128.7 and 150 ppm. Our earlier studies with aromatic amine polymers prepared by enzyme-coupled reactions in an organic solvent have indicated *ortho*- and *para*-carbon-carbon polymerization^{9,10}.

The proton NMR spectrum of the bioengineered polyaniline indicates aromatic protons (at 6.9 to 8.0 ppm) and primary and secondary amine protons at 3.5 ppm and 8.6 ppm, respectively. The ¹H-NMR spectrum along with ¹³C NMR and FTIR spectra

have indicated at least two structurally different types of polyanilines synthesized by enzyme-coupled reactions in organic solvents, an alternating benzoid-quinoid structure, and an *ortho*- and *para*-substituted carbon-carbon and carbon-nitrogen bond structure. These possible structures are indicated in Figs 2A and 2B. It is also possible that the polyaniline product formed by this biocatalysis method could be a complex copolymer of both of the units represented in 2A and 2B. Earlier studies with chemically synthesized polyanilines have indicated only the presence of benzoid-quinoid structures^{14,15}.

Acknowledgement

The authors thank Rosa Linda Bagalawis of the Fiber and Polymer Science Division of US Army Natick RD&E Center for FTIR analyses.

References

- 1 Fritzsche J, *J fur Prakt Chem*, 20 (1840) 454.
- 2 Carlin C M, Kepley L J & Bard A J, *J Electrochem Soc*, 132 (1985) 353.
- 3 Wang B, Tang J & Wang F, *Synth Met*, 13 (1986) 329.
- 4 Chen S A & Lee T S, *J Polym Sci Polym Lett*, Ed 25 (1987) 455.
- 5 Chen S A & Fang W-G, *Macromolecules*, 24 (1991) 1242.
- 6 Osaheni J A, Jenekhe S A, Vanherzeele H, Meth J S, Sun Y & MacDiarmid A G, *J Phys Chem*, 96 (1992) 2830.
- 7 Kibanov A M, *Trends Biochem Sci*, 14 (1989) 141.
- 8 Arnold F H, *Trends Biotechnol*, 8 (1990) 244.
- 9 Akkara J A, Senecal K J & Kaplan D L, *J Polym Sci, Part A: Polym Chem*, 29 (1991) 1561.
- 10 Akkara J A, Salapu P & Kaplan D L, *Polym Mt Sci Eng Preprint Am chem Soc, Div Polymeric and Materials Sci*, 66 (1992) 374.
- 11 MacDiarmid A G, Chiang J C, Richter A F, Somasiri N L D & Epstein A J, *Conducting polymers*, edited by L Alcacer (Reidel, Dordrecht, Holland) 1987, pp. 105.
- 12 Wudel F, Angus (Jr) R O, Lu F L, Allemand P M, Vachon D J, Nowak M, Liu Z X & Heeger A J, *J Am chem Soc*, 109 (1987) 3677.
- 13 Wei Y & Hsueh K, *J Polym Sci Part A: Polym Chem*, 27 (1989) 4351.
- 14 Hjertberg T, Salaneck W R, Lundstrom I, Somasiri N L D & MacDiarmid A G, *J Polym Sci Polym Lett Ed*, 23 (1985) 503.
- 15 Pouget J P, Jozefowicz M E, Epstein A J, Tang X & MacDiarmid A G, *Macromolecules*, 24 (1991) 779.