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Synthesis, characterization, and electrospinning of novel polyaniline–peptide polymers

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ABSTRACT

Aniline–peptide (FLDQV, FLDQVC, Dansyl-FLDQV, Dansyl-FLDQVC, and FLDQV-AMC) mixtures underwent oxidative chemical and electrochemical polymerization in excess of aniline. The products of the chemical polymerization were low molecular weight polymers containing more than 70% peptide. Electrochemically polymerized species polyaniline-FLDQV (PANI-FLDQV) consisted mainly of polyaniline units containing about 10% peptide. The solubility of the latter in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was similar to the camphorsulfonic acid (CSA) doped emeraldine base (PANI-CSA) solubility, however the weight composition of the electrospun fibers produced from the two polymers was significantly different. 2D $^{1}H–^{13}C$ HSQC analyses were employed to analyze the binding between the aniline and peptide moieties. Binding of peptide to polyaniline is reflected by the appearance of extra cross-peaks which display line broadening between the free polyaniline and the free pentapeptide. Peptides may be chemically bonded to the polymer molecules, but they may also act as doping agents to the nitrogen atoms via hydrogen bonding.

1. Introduction

Synthetic polymers, such as polyaniline, polyethylene oxide, and polyethylene glycol, have often been used as matrixes for immobilization of proteins and peptides for catalytic, sensoring, synthetic, or therapeutic purposes [1–3]. While quite useful in many cases, these type of devices often have limited working range restricted by the solubility, structural stability, and pH sensitivity of the components. The search for more effective ways to achieve a synergistic combination of properties leads to the development of protein–polymer conjugates [4–7]. Synthetic strategies include utilization of common chemical reactions (Heck, Suzuki coupling, cycloaddition reactions) [8–10] with polymer-reactive peptides/proteins, or protein/peptide reactive polymers, under mild conditions, in order to render a product with the desired structure and properties. Modification of proteins and peptides occurs through the accessible amino acids and reactions may take place with the carboxyl and amino function as well as with the amino acid side chain [11,12].

Polyaniline, a conductive polymer subject to numerous studies because of easy synthesis, tunable properties, and plenty of potential practical applications, can also be obtained in chiral form in presence of chiral inducers [13–15]. Chiral PANI can be obtained by chemical, electrochemical, or enzymatic polymerization as well as by chiral doping-dedoping procedure [16–18]. Chiral acids, such as (+) or (−) 10-camphorsulphonic acid, have successfully induced chirality in the PANI polymer chains, however DNA, polysaccharide, and cellulose, have never been used for this purpose [19]. From another side, studies have shown that proteins, peptides, and even amino acids, present in the polymerization reaction mixture, can induce chirality and selective recognition sites. Polymerization of aniline monomers in presence of hemoglobin or bovine serum albumin, on DBSA template under mild conditions, produce enantio specificity of PANI probably due to the α-helical structure of

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the proteins [15]. PANI obtained from saturated solutions of L-phenylalanine, using ammonium persulfate as an oxidant, results in chiral PANI of flaky, spherical, and urchin-like morphologies. The latter research assumes also that L-phenylalanine is incorporated in the PANI chains as a dopant [20].

Electrospinning has been utilized to produce fiber mats with various sizes and properties. Polyaniline is notoriously difficult to electrospin since its low solubility in common solvents prevents the formation of solutions with appropriate concentration and viscosity [21]. De-doping with substances, such as camphorsulfonic acid, increases the solubility, however, electrospinning has been reported only in presence of supporting polymers [21–23].

We have previously reported an investigation on the attachment of dioxin selective pentapeptides on polyaniline matrix with or without a linker, and the stability of the chemosensors under different conditions [24]. Some of the peptides used in the study have been previously reported to selectively bind chlorinated toxins [25,26].

The objectives of this research are the following: (1) to explore the chemical and electrochemical polymerization of aniline in presence of penta- and hexapeptides FLDQV, FLDQVC, as well as their Dansyl and AMC labeled derivatives. (2) To produce electrospun fibers from the PANI–peptide polymers and the compare the results with the PANI-CSA doped electrospun mats. (3) To perform preliminary testing of the polymers and the fibers for extraction of chlorinated toxins.

Our hypothesis is that the PANI–peptide polymers and fibers will be promising binding reagents which can be further utilized as solid state extraction tools.

2. Experimental

2.1. Materials and methods

Aniline (99.5%), ammonium persulfate (APS) (98%), phosphate buffer powder, 0.1 M, hydrochloric acid (37%), ammonium hydroxide (28–30%), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, HPLC grade), 1-methyl-2-pyrrolidinone (NMP, spectroscopic grade), and methanol (spectroscopic grade) were purchased from Sigma Aldrich. FLDQV, FLDQVC, FLDQV-AMC, Dansyl-FLDQV, FLDQVC-AMC, and Dansyl-FLDQVC, were obtained from Biomatik, Inc., Ontario, Canada.

The absorption spectra were recorded on Shimadzu 240IPC spectrophotometer. NMR spectra were recorded on a Brucker 700 MHz spectrometer at 298 K. SEM images were produced on Zeiss 1540EsB dual beam Scanning Electron Microscope.

2.2. Synthesis

2.2.1. Chemical synthesis

Emeraldine base and emeraldine salt were synthesized using the procedure of MacDiarmid et al. [13,14]. De-doping with camphorsulphonic acid was done before the electrospinning experiments [22,23].

The chemical polymerization of aniline in presence of non-labeled and labeled peptides, FLDQVC, FLDQV, Dansyl FLDQVC, and FLDQV AMC was carried out at room temperature. The mixture was stirred for 2–4 h. The aniline and the peptides were mixed at 0 °C and the mixtures were kept at room temperature for five days. The peptide was dissolved in methanol before mixing with aniline and 0.1 M APS.

The products of the polymerization reactions were characterized by solid state ^{1}H and ^{13}C NMR, UV/Vis Spectroscopy, Fluorescence Spectroscopy, and Scanning Electron Microscopy (SEM).

A typical polymerization of the peptide–aniline mixtures was done as follows [20]:

400 μL (4.38×10^{-3} mol) of aniline was pipetted into 10 mL of 0.1 M ammonium persulfate (APS). The mixture was placed in an ice bath, stirred, and the peptide solution in 1.5–2 mL methanol was added. The peptide:aniline molar ratio was 1:25–1:30. After 5 days at room temperature, the dark brown precipitate was filtered, rinsed with distilled water and acetone, and dried in the air at room temperature. The reaction yield was 30–40%.

2.2.2. Electrochemical synthesis

The 100 mL electrochemical cell was equipped with a platinum foil counter electrode, and a KCl saturated calomel reference electrode (SCE), against which all potentials were measured. The working electrode was a rotating platinum disk (RDE, from Pine Instruments), 5 mm diameter, mounted in a Pine AFMSRCE rotator and speed controller. Potential ramps were generated with the aid of a Pine AFTP1 Wavenow potentiostat, and the resulting voltammograms were recorded using Aftermath software. The electrode was polished with 0.05 μm alumina (Buehler), sonicated for 30 s, rinsed with water, and then dried with a stream of nitrogen.

Adsorption of polyelectrolytes onto the RDE at 300 rpm was done with the aid of a robot (StratoSequence V, nanoStrata Inc.). The polymer deposition solutions contained 100 mg aniline (1.0×10^{-3} mol) in 100 mL 0.25 M HCl. FLDQV (peptide:aniline 1:125–1:15.6 molar ratio) was dissolved in minimum amount of methanol and added to the mixture. Between polyelectrolyte depositions (5 min each), there were three rinses with deionized water (1 min each). Rinse and polymer solution volumes were approximately 50 mL each. The thickness of the PANI was measured in a separate experiment using a 1 in. diameter single side polished silicon wafer and determined using a Gaertner Scientific L116S autogain ellipsometer with 632.8 nm radiation at 70 incident angle.

Cyclic voltammograms (CVs) at the RDE were performed by sweeping the potential at a 20 mV s^{-1} scan rate in the range of 500–250 mV vs SCE, rotation rate 1000 rpm. Purging the solution for 10 min with Ar was necessary to remove dissolved oxygen; a blanket of Ar was maintained during the experiments [27].

2.3. Electrospinning

EB was mixed in a mortar with CSA in 1:2 ratio according to previously reported procedure. The dedoped PANI-CSA was mixed with HFP, sonicated for 30 min, stirred overnight, and filtered through 20 μm syringe filter to obtain 5% solution. A 20% solution of gelatin in HFP was prepared similarly. Equal volumes of the two solutions were mixed so that the final concentrations of PANI-CSA and gelatin in the mixture were 2.5% and 10% correspondingly. 5 mL of this mixture was electrospun at 14 kV, using 18 gauge needle, delivery rate of 0.8 mL/min, and 12 cm distance from the cathode. Same conditions were used to produce electrospun fibers from the electrochemically polymerized PANI-FLDQV/gelatin mixtures.

3. Results and discussion

The absorption and emission spectra of the peptides and polymerization products were collected in N-methyl pyrollidinone (NMP) which has an optical cut-off at 285 nm. The spectra are shown in Figs. 1 and 2. Dansyl-FLDQVC peptide exhibits a broad absorption band at 346 nm. Two absorption bands were observed in the spectrum of the polymerized product. The long-wavelength absorption maximum undergoes slight hypsochomic shift (λ_{max} 338 nm) and a new absorption band appears at 306 nm. The former is due to the presence of the dansyl chromophore, while the latter can be assigned to the π–π^* transitions of the polyaniline backbone. It is possible that polyaniline has another absorption transition