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Current Applied Physics 4 (2004) 347–350
Current Applied Physics
Physics, Chemistry and Materials Science
www.elsevier.com/locate/cap

The antioxidant activity of conducting polymers in biomedical applications

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Abstract

The radical scavenging ability of soluble conducting polymers has been examined using the DPPH assay in comparison with phenolic antioxidant compounds present in the diet. The reducing strength was also determined by voltammetry at a carbon electrode both in an aqueous pH 7.0 buffer, and in methanol as used for the DPPH assay. The conducting polymers were shown to be good reducing agents and effective scavengers of free radicals, with 2–4 DPPH radicals being reduced for each aniline or pyrrole unit on the polymer chains. The significance of this antioxidant capacity for the application of conducting polymers as biomaterials is considered.

PACS: 72.80.Le; 73.61.Ph; 81.05.Lg; 82.45.+z
Keywords: Antioxidant; Polyaniline; Polypyrrole; Conducting polymer; DPPH; Voltammetry

1. Introduction

A range of biomedical applications for conducting polymers are currently being considered, including the development of artificial muscles, controlled drug release, and the stimulation of nerve regeneration [1]. Low cytotoxicity and good biocompatibility are evident from the growth of cells on conducting polymers and from the low degree of inflammation seen in test animals over a period of several weeks [2]. Given that conducting polymers are redox active, and can shuttle between reduced and oxidized forms, potential interactions of the polymers with biological media need to be carefully considered.

In this paper the ability of conducting polymers to act as reducing agents and scavenge free radicals, and in this sense to act as antioxidants, is considered. Phenolic antioxidants in the diet are thought to offer protection against cardiovascular diseases and cancers where the onset of the disease involves oxidative damage caused by excessive levels of free radicals [3]. Polyaniline and substituted polyanilines have already been examined for their use as antioxidants in rubber materials [4]. However, their antioxidant ability in biological media needs to be examined to assess their likely activity in biomedical applications [5].

For this purpose we have used the capacities of commercially available solutions of polyaniline, poly(anilinesulfonic acid) and polypyrrole (from Sigma–Aldrich) to scavenge the stable α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical in methanol, a procedure widely used to test the antioxidant properties of molecules [6]. The reducing strength of the conducting polymers in a neutral pH 7.0 phosphate buffer, and in methanol, has also been determined by voltammetry at an inert carbon electrode, to give the formal (oxidation–reduction) potentials. The DPPH results and formal potentials have also been compared to those of aniline and pyrrole monomers, and to known antioxidant compounds present in the diet.

2. Experimental

Aniline (Riedel–de-Haën, 99.5%), pyrrole, *N*-methylaniline, *o*-methoxyaniline (Lancaster, 99%) (Chart 1), were freshly distilled and stored in the dark under N_2.

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doi:10.1016/j.cap.2003.11.045

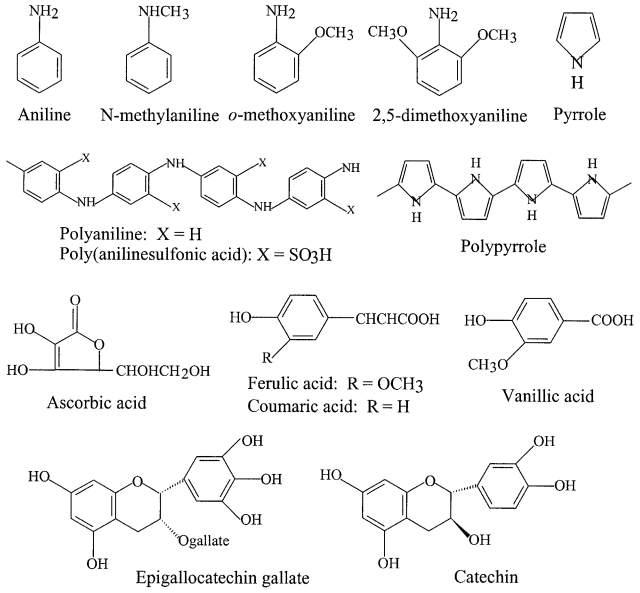

Chart 1. Structures of the compounds examined in this paper.

Solid 2,5-dimethoxyaniline (99%) and aniline-5-sulfonic acid (95%) were obtained from Acros. Solutions were made up using Milli-Q grade water, and all chemicals were of analytical reagent grade. Aqueous solutions of conducting polymers were obtained from Sigma–Aldrich, including 20 wt% short-chain polyaniline grafted to lignin, specified as redox active up to pH 9; 5 wt% poly(anilinesulfonic acid); and 5 wt% polypyrrole. The phenolic antioxidants catechin, epigallocatechin gallate, ferulic acid, vanillic acid, coumaric acid, along with ascorbic acid, were obtained from Sigma–Aldrich.

The DPPH assay for radical scavenging activity was based on the methodology of Neill et al. [7]. 100 μl of a methanolic solution of each test compound were added to 1.5 ml of a 72 μM solution of the stable α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical in methanol, vortexed, and left to stand for 30 min at room temperature. The absorbance of the reaction mixture was then measured at 516 nm using a Schimadzu UV-1240 spectrophotometer. The experiments were repeated at different concentrations to determine the amount required to scavenge 50% of the DPPH radicals (IC_{50} values).

Linear sweep voltammograms were recorded using a BioAnalytical Systems (BAS) 100A electrochemical analyser with BAS C2 cell stand at 100 mV s^{-1}, using a platinum coil counter electrode. The working electrode was a 3 mm glassy carbon electrode (BAS M-2012), polished between runs on alumina powder (BAS PK-4 polishing kit). The voltammograms were taken in either neutral pH 7.0 phosphate buffer containing 65% w/v 0.05 M disodium hydrogen phosphate and 35% w/v 0.05 M sodium dihydrogen phosphate, using an aqueous Ag/AgCl reference electrode (207 mV), or in methanol containing 0.1 M $LiClO_4$ supporting electrolyte and a nonaqueous Ag/Ag^+ (0.01 M) reference electrode (BAS MF-2062). The background response for the carbon electrode in the phosphate buffer or the methanol solution was subtracted to produce the response due to the test compound (in the 0.1–1 mM concentration range). Formal potentials were estimated as the potential at 3/4 of the peak height, where a clear anodic peak was formed, or in other cases about 100 mV after the current began to exceed that of a solvent blank.

3. Results

3.1. Antioxidant activity measured using the DPPH assay

The number of coloured DPPH radicals, which absorb strongly at 516 nm, was seen to decline as more of each of the three soluble conducting polymers was added. This is shown in Fig. 1 for the soluble 20% w/v polyaniline grafted to lignin, where 0.01 μl of the solution (0.002 μg of polyaniline, or 2.2×10^{-8} moles of aniline units) was sufficient to lower the level of 1.5 ml of 72 μM of DPPH radicals in methanol by close to 50%. This equates to the reduction of 2.4 DPPH radicals for each aniline unit of the polymer (Table 1). Soluble 5% w/v polypyrrole and poly(anilinesulfonic acid) produced similar results, with values in the range of 3–4 DPPH radicals reduced per monomer unit. By contrast, the aniline monomer reacted less readily, and a ratio of 170 aniline molecules per DPPH radical was required to lower the level of DPPH radicals by 50%. On the other hand, with *o*-methoxyaniline, the ratio of monomer to DPPH radical was close to 1–1 (Table 1).

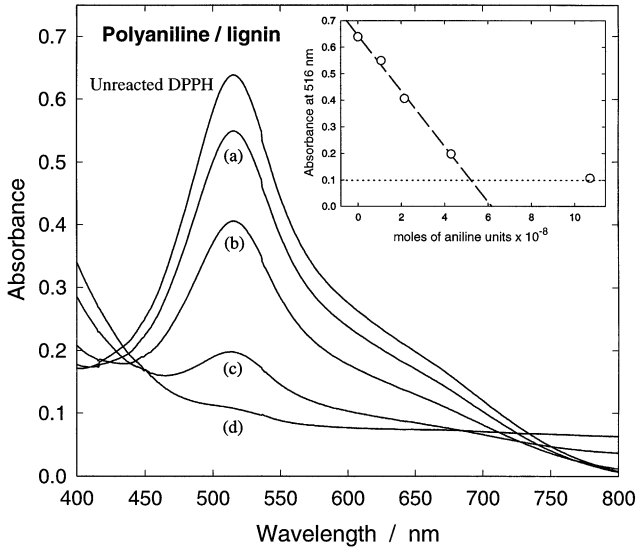

Fig. 1. Visible spectra of DPPH radical in methanol after 30 min exposure to nil, (a) 0.005 μl, (b) 0.01 μl, (c) 0.02 μl, and (d) 0.05 μl of a 20% w/v solution of short-chain polyaniline grafted to lignin produced by Sigma–Aldrich.

Table 1
Electrochemical formal (oxidation–reduction) potentials ($E^{o\prime}$) at a carbon electrode, and DPPH radical scavenging abilities, of conducting polymers, their monomers, and of selected phenolic antioxidants

Compound	$E^{o\prime}$/mV (Ag/AgCl) aqueous at pH 7.0	$E^{o\prime}$/mV (Ag/Ag$^+$) in methanol	DPPH·: compound
Conducting polymers			
Polyaniline/lignin	150	400	2.4:1
Poly(anilinesulfonic acid)	150	300	3.7:1
Polypyrrole	450 (150 at Pt [5])	400	3.4:1
2,5-dimethoxyaniline		265	
o-Methoxyaniline	500	360	1.1:1
N-methylaniline		500	
Aniline	700	570	1:170
Pyrrole	700	700	
Aniline-2-sulfonic acid		770	
Free radicals			
DPPH·	340 [9]	500	
ROO·	800 [10]		
OH·	2100 [10]		
Phenolic antioxidants			
Ascorbic acid	75 [10]	80	2:1 [6,11]
Epigallocatechin gallate	90	310	12.5:1 [12]
Catechin	190	400	3.8:1 [11] 5:1 [12]
Ferulic acid	410	530	1.2:1 [6,11]
Vanillic acid	570	700	0.48:1 [11] 0.08:1 [6]
Coumaric acid	580	800	0.38:1 [11] 0.01:1 [6]

These results can be compared to the very efficient radical scavenging of ascorbic acid and phenolic antioxidants such as catechin and epigallocatechin gallate (with easily oxidizable *ortho*-diphenol or triphenol groups) (Table 1), the more moderate effectiveness of ferulic acid, and weaker radical scavenging of vanillic and coumaric acids (with more isolated phenol groups—see Chart 1).

3.2. Reducing strength of the soluble conducting polymers, monomers and phenolic compounds

Voltammetry at a carbon electrode was used to determine the potentials at which the soluble conducting polymers and various monomers were oxidized, both in a neutral pH phosphate buffer, typical of conditions of biological pH, and in methanol, the solvent in which the DPPH assay was conducted. As seen in Table 1 and Fig. 2, relatively high potentials were required to oxidize aniline and pyrrole monomers, consistent with the low reactivity of aniline with DPPH radicals. Substitution with methyl or methoxy-groups served to lower the potential for oxidation into the same range as for ferulic

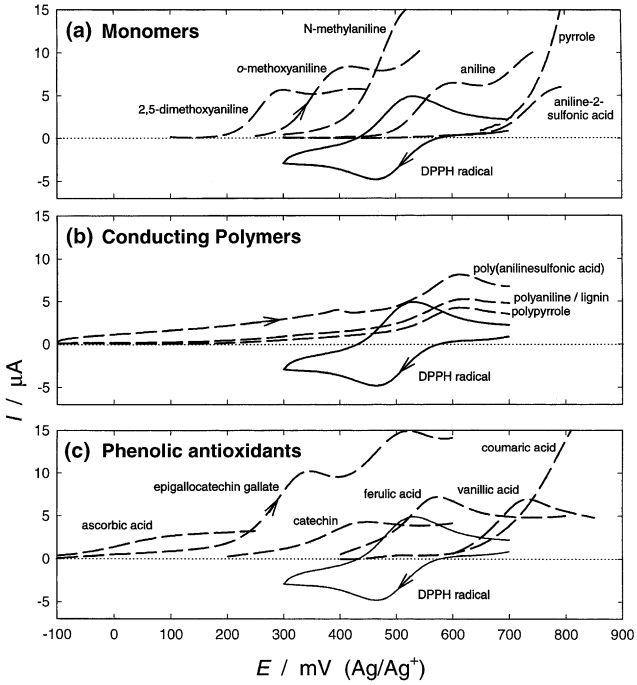

Fig. 2. Linear sweep voltammograms of (a) monomers, (b) soluble conducting polymers, and (c) phenolic antioxidants, taken at a 3 mm carbon at a scan rate of 100 mV s^{-1} in methanol containing 0.1 M $LiClO_4$. In each case a cyclic voltammogram for the DPPH radical, scanned initially from 700 mV in the cathodic direction, has been included.

acid and catechin, leading to moderate DPPH· scavenging. On the other hand, in methanol the sulfonated aniline oxidized at a higher potential than aniline.

In the aqueous pH 7 phosphate buffer, the three soluble conducting polymers were seen to oxidize at potentials much lower than their monomers (Table 1), a well established trend [8]. In methanol the conducting polymers were oxidized from a potential of 300 mV (Ag/Ag$^+$) or lower (Fig. 2). The potentials for the conducting polymers lay quite close to those of the phenolic antioxidants catechin and epigallocatechin gallate (present in teas and red wines), which had very low formal potentials in both solvents, while ascorbic acid was the strongest reducing agent.

The DPPH radical itself has been reported to have a formal potential at pH 7.0 of 340 mV (Ag/AgCl) [9], while in methanol a value of 500 mV (Ag/Ag$^+$) was obtained (Table 1 and Fig. 2). Many of the radicals which are of biological interest, such as peroxyl radicals (ROO·) and the hydroxyl radical (OH·), exhibit much higher formal potentials and would likely be reduced by any of the compounds listed in Table 1 [10]. Compounds with a formal potential similar to, or lower, than that of the DPPH radical can readily reduce DPPH· in the methanol based assay. The low formal potentials for the soluble conducting polymers, for *o*-methoxyaniline, and for ascorbic acid, epigallocatechin gallate and catechin,

are consistent with their efficient scavenging of DPPH radicals. On the other hand, the higher potentials for the various aniline or pyrrole monomers, and for phenolics with isolated phenol groups, match their lower reactivity with DPPH·.

4. Discussion

The electrochemical reducing strengths of the soluble conducting polymers examined in this paper are very similar to those of phenolic antioxidants present in various fruits and beverages. This reducing ability is matched by efficient scavenging of DPPH radicals, with between 2 and 4 radicals scavenged per aniline or pyrrole monomer unit during the 30 min test period of the DPPH assay. These results show the potential for conducting polymers to be effective antioxidants when present in biological media.

We are currently investigating the kinetics and mechanisms of the reaction between conducting polymers and the DPPH radical. While the conducting polymer will inevitably be oxidized as part of this process, the product may either involve positively charged centres on the polymer backbone, balanced by an anionic form of DPPH or other solution anions, or should DPPH· extract a proton from the polymer to form DPPHH, other polymer species will result. The fact that multiple DPPH radicals are neutralized for each aniline or pyrrole unit on the conducting polymers indicates that further reaction pathways may be involved whereby the polymer dissipates charge to the surrounding solvent, or else polymer cross-linking reactions and other processes may follow.

The antioxidant activity of conducting polymers such as polyaniline and polypyrrole needs to be considered when assessing their impact as biomaterials. This property may even be beneficial, particularly in situations in which disease states may lead to excessive levels of free radical species, which the conducting polymers may be able to remove.

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