Antioxidant Properties of Layer-by-Layer films on the Basis of Tannic Acid

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Tannic acid, a polyphenol of natural origin, assembled in alternation with polyelectrolytes via the layer-by-layer method retains its ability to scavenge free radicals. Kinetics of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation-radical bleaching by tannic acid/polyelectrolyte films depend on multilayer architecture, while total radical scavenging capacity depends on the amount of adsorbed TA.

Layer-by-layer (LbL) assembly allows to create thin layers of components (polyelectrolytes, nanoparticles, biomolecules, etc.) with target properties on different templates.[1] Tannic acid (TA), a polyphenol of natural origin, can be used as a structural block in the assemblies in alternation with positively charged polyelectrolytes. Multilayers on flat surfaces and microcapsules with controllable permeability have been developed.[2]

Phenols possess high ability to scavenge free-radicals and inhibit radical-induced oxidation of molecules.[3–7] Deposited as an outermost layer on the surface of the devices, layers of polyphenol can significantly inhibit their damage by free radicals trapping them before they can reach inner layers based on biomolecules or polyelectrolyte capsules. The validity of a similar approach has been recently demonstrated by the example of hemoglobin/polyelectrolyte LbL film protection by catalase layers under hydrogen peroxide treatment.[1c]

Tannic acid (TA, pentagalloyl glucose), sodium poly(styrene sulfonate) (PSS), poly(allylamine hydrochloride) (PAH), horseradish peroxidase (HRP), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and Trolox were purchased from Sigma-Aldrich.

$(PAH/TA)_n$ ($n = 0.5$–6.5) and $(HRP/PSS)_5/PAH$ films were assembled at pH 6.5 on glass slides by sequential dipping in 3.0 mg/mL solutions of the components as in Ref. 2. A TA or PSS layer was adsorbed on $(HRP/PSS)_5/PAH$ films from 0.1 mg/mL solutions.

Two systems based on the cation-radicals of ABTS ($ABTS^{+\bullet}$) were used to study specificity of radical reactions with LbL-assembled layers containing polyphenol.

In the first experimental arrangement, an $ABTS^{+\bullet}$ solution in DI water with an initial absorbance of 1.2 ± 0.1 at 734 nm ($\varepsilon = 1.4 \times 10^4\,M^{-1}\,cm^{-1}$) was prepared as proposed in Ref. 3. The radical bleaching in time was followed after an aliquot of a TA solution was added or a film was immersed in the solution.

The second system is based on $ABTS^{+\bullet}$ production by HRP/PSS films. The $(HRP/PSS)_5/PAH$ films with a PSS or TA layer on the top were immersed in an ABTS–H_2O_2 solution, and color appearing in the solution was observed.

TA shows a mixed scavenging activity toward $ABTS^{+\bullet}$. A short fast period completed within 20 s after adding TA is followed by a long slow scavenging stage (data not shown). Biphasic kinetics have been observed for the $ABTS^{+\bullet}$ reaction with TA and several other antioxidants, among them complex flavonoid compounds, and attributed to scavenging by polyphenol oxidation products formed on early stages of the reaction.[4] Under the same conditions, for Trolox used as a standard, no slow scavenging period was observed. The estimated radical scavenging activity (RSA) of Trolox, which shows how many $ABTS^{+\bullet}$ radicals react with one inhibitor molecule, is 1.82 ± 0.37 and consistent with previously reported data (RSA $= 2$).[5] For TA, the RCA value reaches 23.2 ± 3.2 after 20 min. Taking into account, that for several polyphenols (including gallic acid), around 1.92 ABTS cation radicals react with one –OH group[5] one can calculate that after 20 min the reaction is completed to about 80%.

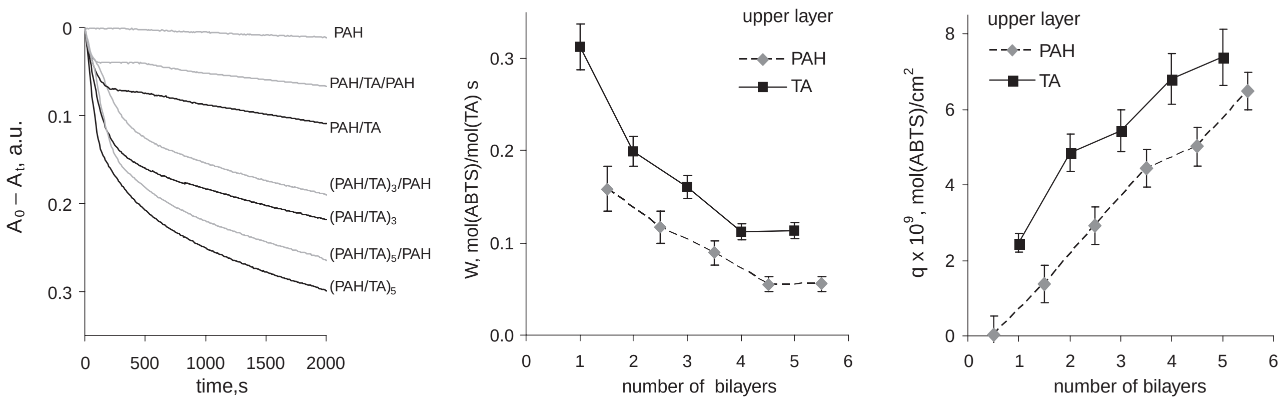
The initial reaction rate of ABTS decolorization is proportional to TA concentration in solution (data not shown). The calculated second-order constant for the reaction of TA with ABTS is $(9.7 \pm 0.8) \times 10^3\,M^{-1}\,s^{-1}$. For several other phenols, the value is reported to be within 10^2–$10^3\,M^{-1}\,s^{-1}$.[6]

In TA/PAH films, the reaction of TA with $ABTS^{+\bullet}$ cation-radicals retains biphasic characteristics (Figure 1a). The initial $ABTS^{+\bullet}$ scavenging for TA/PAH films is more than 3 times lower than in solutions (0.76 ± 0.10 mol $(ABTS^{+\bullet})$/mol(TA)·s), and decreases with increasing number of TA layers (Figure 1b). At the same time, the estimated values of RSA for TA/PAH films after 30 min of the reaction are 26.1 ± 0.6 regardless of the number of bilayers.[8] Apparently, upper TA layers readily consume the cation-radicals preventing them from reaching inner layers of the film on initial stages of reaction. The later participate in the reaction after TA in the upper layers is mostly spent.

RSA of the films with a TA outermost layer is somewhat higher than that for PAH-terminated films with the same number of TA layers (Figure 1c), and corresponding curves increase more sharply (Figure 1a). The nature of the outermost layer in LbL assembled multilayers obviously influences the film properties.[1] It has been shown that a PAH layer deposited on top of a TA layer increases local pH in the vicinity of LbL films and results in an increase of TA dissociation degree.[2]

At higher pH, polyphenols (polygalloyl glucose, procyanidine, etc.) show higher RSA values and react faster with ABTS cation-radicals.[4,7] Kinetics of $ABTS^{+\bullet}$ bleaching on variously terminated films do not coincide with those observations made in the absence of polyelectrolytes that form a reversible complex with tannic acid. Deposition of a PAH layer shifts the equilibrium to the complex, slowing the reaction of tannic acid with the cation-radicals. Inhibition of the reaction of $ABTS^{+\bullet}$ with a polyphenol by a protein forming strong complexes with the compound has been reported.[4]

An outermost TA layer on a $(HRP/PSS)_5/PAH$ film significantly slows down the initial rate of color appearance in the surrounding solution containing ABTS and H_2O_2 (Figure 2). Since



a b c

Figure 1. ABTS$^{+\bullet}$ bleaching by TA/PAH films with different number of layers: a) kinetic curves, b) initial rates of radical bleaching, and c) amount of radicals decomposed by 1 cm^2 of the films after 30 min.

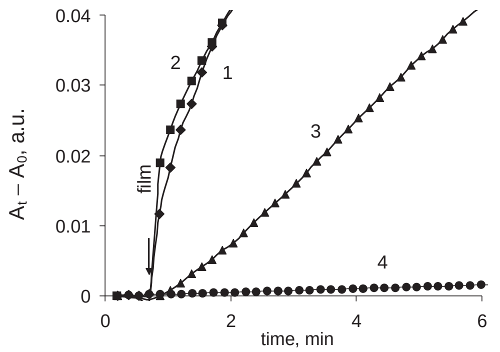


Figure 2. ABTS$^{+\bullet}$ color appearance in a 1.0 mM ABTS–0.1 M H_2O_2 solution after immersion of a (HPR/PAH)$_5$/PAH film: 1—without additional layer, 2—with PSS layer, 3—with TA layer, 4—bare glass template.

a PSS layer deposited on the top of a (HRP/PSS) film does not affect ABTS$^{+\bullet}$ formation, the influence of the TA layer is rather related with its activity towards ABTS$^{+\bullet}$, but not with diffusion limitations.

No prominent inhibition period was observed for the films. The initial rates of ABTS$^{+\bullet}$ appearing are 0.4×10^{-11} and 1.9×10^{-11} mol/s·cm^2 for the films with and without the TA layer. Considering the TA amount adsorbed to be around 7×10^{-11} mol/cm^2,[8] we estimated the rate of cation-radical consumption as 0.2 mol(ABTS$^{+\bullet}$)/mol(TA)·s, which is comparable with the maximal values found for TA/PSS multilayers. In LbL assemblies with polyelectrolytes, TA behaves as an effective scavenger of the cation-radicals.

At the same time, in HRP-ABTS-H_2O_2 solutions,[9,10] the inhibition coefficient estimated from the dependence of the induction period on TA concentration according to the theory of inhibitors[10] is equal to 3.0 (data not shown). Only three radicals react with one TA molecule under these conditions indicating a complex mixed mechanism of inhibition.[10] Immobilization of HRP and TA in LbL film spatially separates them and apparently excludes some of the reaction paths in which TA acts as a substrate for HRP.

Polyphenols assembled in alternation with polyelectrolytes via LbL method retain their ability to scavenge free radicals. The results suggest that polyphenol multilayers positioning as organized protective shells on the surface of biodevices or compacted drug forms may inhibit or diminish free-radical damage of encapsulated compound. By varying number of deposited layers, antioxidant properties of the assemblies can be changed.

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