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The effect of DOPA hydroxyl groups on wet adhesion to polystyrene surface: An experimental and theoretical study

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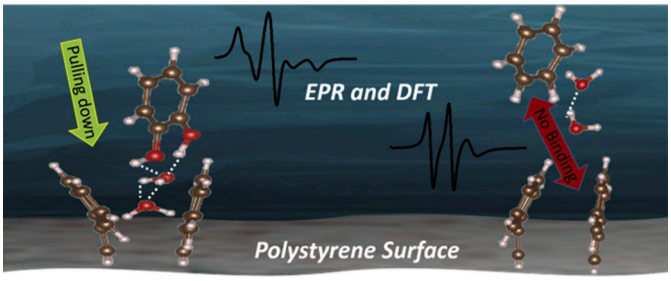
HIGHLIGHTS

- Hydroxyl groups in DOPA play a crucial role on the adhesion to wet styrene surface.
- DOPA or tyrosine ended PEG covered the styrene surface but not with phenylalanine.
- Without water, catechol has the lowest adhesion ability to the styrene surface.
- EPR spectroscopy provides the degree of surface coverage of nanoparticles in water.
- AIMD simulations with and without water support the EPR results.

GRAPHICAL ABSTRACT



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ABSTRACT

Mussels wet adhesive performance has been arousing curiosity for a long time. It is found that 3,4-dihydroxyphenylalanine (DOPA) is responsible for adhesive properties of mussels. Despite a large body of research characterizing the interactions DOPA with hydrophilic surfaces, relatively few works have addressed the mechanism of interactions with hydrophobic surfaces. The benzene ring of DOPA is the main attributor to the adhesion on hydrophobic polystyrene (PS) surface. However, here we showed that two hydroxyl groups of catechol have also effects on wet adhesion. We studied wet adhesive properties of DOPA, tyrosine and phenylalanine functionalized PEG polymers, PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$, PEG-(N-Boc-L-Phenylalanine)$_4$, on spin labeled PS nanobeads (SL-PS) by electron paramagnetic resonance (EPR) spectroscopy. Surface coverage ratio of SL-PS upon additions of PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$ showed that SL-PS was covered with 70%, 50% and 0%, respectively. This showed that spontaneous wet adhesion on PS increases with the number of amino acids hydroxyl groups. This is also supported with the density functional theory (DFT) energy calculations and ab-initio molecular dynamics (AIMD) simulations. In water, interactions between water molecules and hydroxyl groups on the catechol induce catechol adhesion via π-π stacking between the catechol and double styrene rings which were already tilted out with water.

1. Introduction

Wet adhesion properties of marine mussels have inspired scientists to obtain adhesive materials work underwater [1,2]. In aqueous solution, adsorbed water molecules (hydration layers) hinders polymer adhesion to wet surfaces. Pre-adsorbed water molecules obstruct molecular

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interaction and hence the stable bonds formation between the polymer and the surface. However, marine mussels have remarkable ability to stick to various wet surfaces using different types of mussel foot proteins (Mfps) [3]. Most Mfps contain posttranslationally modified tyrosine amino acid called 3,4-dihydroxyphenylalanine (DOPA), through to different degrees (0.1–30 mol%) [4].

It has been shown that catechol moiety of DOPA is responsible for both adhesive and cohesive properties of mussels [5,6]. A benzene ring and two neighboring hydroxyl groups provide DOPA to interact with different types of surfaces via different types of interactions, ranging from weak dispersion forces to covalent bonds [7]. Several interaction mechanisms of DOPA with different surfaces have been reported, including hydrogen bonding, hydrophobic interactions, electrostatic interactions, π-π stacking, cation-π interactions and metal-complexation [8]. DOPA has a strong affinity for hydrophilic surfaces, bidentate hydrogen bonding by DOPA is the main contributor to DOPA adhesion to mica and silica [8]. However, studies of surface forces apparatus (SFA) which is used to measure the physical forces between surfaces showed that hydrophilic Mfps (Mfp-1, Mfp-3F, and Mfp-5) adhere 10 times more strongly to hydrophobic surfaces (CH_3- terminated) than to hydrophilic surfaces (OH- terminated) [9]. Moreover, the water molecules within the strong hydration layers of hydrophilic surfaces prevent DOPA approaching to the surface [10,11]. For example, DOPA-rich Mfp-3 and DOPA conjugated 4-armed polyethylene glycol (PEG) polymers (PEG-$(DOPA)_4$) cannot adhere to surface of hydrophilic silica nanobead under force-free conditions. But, they can spontaneously adhere to hydrophobic surface of polystyrene (PS) nanobead in water. In addition, attaching anionic surfactant molecules, sodium dodecyl sulfate (SDS), to amine functionalized silica surface disturbs the hydration layers of silica, and thus PEG-$(DOPA)_4$ is able to adhere to wet silica surface [12].

Although π-π stacking is strong enough to establish the adhesion between DOPA and PS [2], the mechanism of DOPA adhesion to PS surface has not been well understood. In the previous study, another aromatic amino acid, tryptophan (Trp), was conjugated with 4-armed PEG polymers [11]. It has been shown that PEG-$(Trp)_4$ did not adhere to PS surface in water under force free conditions, although it is more hydrophobic than DOPA and also it has an aromatic benzene ring. This result showed that hydrophobic interactions and π-π stacking between tryptophan and styrene rings are not strong enough to achieve adhesion in water. In addition to these interactions, hydroxyl groups of DOPA could be effective for a durable adhesion in water. Interactions between hydroxyl groups of DOPA and adsorbed water molecules in the adhesion interface could help weaken the hydration barrier. Therefore, DOPA conjugated polymer could be more interactive with the surface molecules. In order to understand the effect of DOPA hydroxyl groups on adhesion to the PS surface, tyrosine having a single hydroxyl group or phenylalanine without any hydroxyl group were attached to the 4-armed PEG polymers, and their adhesion properties were studied both experimentally and theoretically (Fig. 1).

Here, the ^1H NMR and UV–Vis spectroscopic techniques were used to characterize the modification of PEG polymers with DOPA, tyrosine and phenylalanine. As an alternative technique, continuous wave (cw) electron paramagnetic resonance (EPR) spectroscopy was used to study the wet adhesion of each DOPA, tyrosine or phenylalanine functionalized 4-armed PEG polymers to the polystyrene surface. EPR spectroscopy in combination with spin labeling has already been used in the previous surface adhesion studies [10–12]. Dynamics behaviors of spin labels (SL) on the PS surface characterized with rotational correlation times determine the EPR line shapes. Shortly, spin labels that have relatively free motions yield sharper signals with shorter rotational correlation times, instead spin labels that have more restricted motions yield broader signals with longer rotational correlation times [13,14]. Rotational correlation time of SL on the PS surface increases upon polymer adhesion due to the restricted slow motion of covered SL. Consequently, a new type of spectrum with a slow motion can be detected simultaneously with the spectrum of uncovered SL on the surface. Hence, the covered fraction of surface can be detected upon adhesion of the polymer by the EPR spectroscopy. This allows making comparison between the adhesive properties of DOPA, tyrosine and phenylalanine. To further investigate this phenomenon, density functional theory (DFT) energy calculations and DFT based molecular dynamics (AIMD) simulations were performed. The effect of hydroxyl groups on adhesion of DOPA was analyzed by using catechol, phenol or benzene with double styrene rings in the presence of two water molecules. Styrene-styrene interactions might be effective for the DOPA adhesion. Therefore, for a reliable simulations of the surface we studied double styrene rings with catechol, phenol and benzene molecules. It is discovered both experimentally and theoretically that hydroxyl groups on the benzene rings enhance the spontaneous adhesion to styrene surface under water.

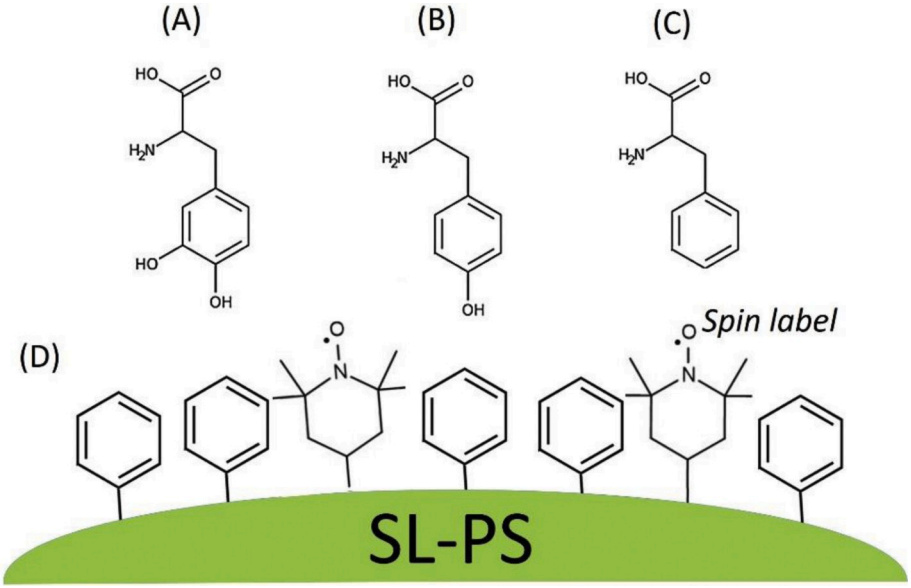

Fig. 1. Molecular structures of (A) L-DOPA, (B) L-Tyrosine, (C) L-Phenylalanine and (D) colloidal spin labeled polystyrene surface (SL-PS).

2. Experimental and computational methodology

The syntheses of N-Boc-L-DOPA, PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$ were prepared as described in published procedures [11,15,16]. N-Boc-L-Tyrosine and N-Boc-L-Phenylalanine were provided from Sigma-Aldrich. Spin labeling of polystyrene was done according to the published procedure [10].

2.1. Synthesis of N-Boc-L-DOPA

L-DOPA (0.4 mmol) and triethylamine (86 μL) were dissolved in water:dioxane (1:1) mixture (800 μL) in an ice bath (0 °C). 0.45 mmol di-*tert*-butyl dicarbonate (Boc) dissolved in dioxane (400 μL) was added into the mixture containing L-DOPA and triethylamine, and stirred at 0 °C for 30 min. Additionally, the mixture was stirred at 25 °C for 17 h. At the end of the reaction, the mixture was extracted with ethyl acetate (50 mL) and the pH of the organic phase was adjusted to 1.0 by HCl and back extracted with ethyl acetate (50 mL) for 3 times. Combined organic phases were dried over Na_2SO_4. Organic solvent was evaporated to afford N-Boc-L-DOPA as a brown color which was used in the next step without any purification (84% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.87 (d, 1H), 6.57 (s, 1H), 6.43 (d, 1H), 3.92 (br, s, 1H), 2.77-2.60 (m, 2H), 1.30 (s, 9H).

2.2. Synthesis of PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$

N-Boc-L-DOPA (or N-Boc-L-Tyrosine, N-Boc-L-Phenylalanine) (23.9 mg, 80 mmol), PEG-$(NH_2)_4$ (10 kDa) (97 mg, 9.7 × 10^{-3} mmol), 1-hydroxybenzotriazol (HOBT) (17.3 mg, 0.128 mmol) and triethylamine (17.6 μL) were dissolved in a mixture of DCM (460 μL) and DMF (460 μL) at 25 °C. 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (29.6 mg, 0.078 mmol) and DCM (460 μL) were added into the mixture and stirred at 25 °C under argon atmosphere for 5 h. At the end of the experiment, ninhydrine test was applied to control the remained free primary amine left on the PEG. 2–3 drops of product were dissolved in DCM (1 mL) and 2–3 drops of ninhydrine solution was put into the solution. The mixture was stirred at 50 °C for 30 min, the test result was negative. The crude product was washed with saturated sodium chloride solution (50 mL), $NaHCO_3$ (5% w/mL) solution, HCl (1 M) solution (50 mL), and distilled water (50 mL). The organic phase was dried over Na_2SO_4 and the product was precipitated in cold diethyl ether for 3 times. The yields of products and ^{1}H NMR results are follow:

PEG-(N-Boc-L-DOPA)$_4$: 96% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.84 (s, 4H), 6.79-6.55 (m, 12H), 3.96 (br, s, 4H), 3.56-3.38 (m, 896H), 2.55–2.70 (m, 8H), 1.28 (s, 36H).

PEG-(N-Boc-L-Tyrosine)$_4$: 70% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.84 (s, 4H), 7.05 (d, 8H), 6.59 (d, 8H), 4.00 (br, s, 4H), 3.47-3.38 (m, 896H), 2.75-2.57 (m, 8H), 1.27 (s, 36H).

PEG-(N-Boc-L-Phenylalanine)$_4$: 84% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.91 (s, 4H), 7.20 (m, 20H), 4.09 (br, s, 4H), 3.47-3.36 (m, 896H), 2.88-2.66 (m, 8H), 1.26 (s, 36H).

2.3. Preparation of spin labeled polystyrene (SL-PS) nanobeads

For spin labeling of PS surface, a nitroxide type 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo) based radical was used. 250 μL 4-carboxy Tempo (10 mM) dissolved in 0.2 M MES buffer (pH 3.0) was mixed with 100 μL amine-modified polystyrene nanobead (Sigma Aldrich, 50 nm particle size) in the presence of a cross linker, 38 mM, 90 μL 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Thermo Scientific) for one day at room temperature. Excess EDC and 4-carboxy Tempo were washed out several times with MES buffer at pH 3.0.

2.4. EPR measurements and calculations of surface coverage

For EPR measurements, 180 mg/mL of PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ or PEG-(N-Boc-L-Phenylalanine)$_4$ dissolved in 0.2 M MES buffer at pH 3.0 were mixed with the same volume of SL-PS solution in 0.2 M MES buffer at pH 3.0. After a few minutes, 7 μL of mixture was transferred into the quartz capillary tubes for EPR measurements. A CMS 8400 (Adani) X-band EPR benchtop spectrometer was used for room temperature experiments. EPR spectra were simulated using a Matlab based Easyspin 4.5.5 software package [17].

EPR spectra of SL-PS after addition of PEG-(N-Boc-L-DOPA)$_4$ and PEG-(N-Boc-L-Tyrosine)$_4$ were simulated using individual spectral simulations of uncovered (S1) and covered (S2) spin labels. The complete simulations of experimental results were obtained by combining the individual components, (1) and (2) with appropriate numbers $x_{(1)}$ and $x_{(2)}$ as given by:

$$\text{Measured spectrum} = x_{(1)}S1 + x_{(2)}S2 \quad (1)$$

The surface coverage percentage is calculated by the formula as given by:

$$\%\text{Covering} = \text{Covered Area} / (\text{Covered Area} + \text{Uncovered Area}) \quad (2)$$

where covered area is calculated from the multiplication of area under S1 with $x_{(1)}$, and uncovered area is calculated from the multiplication of area under S2 with $x_{(2)}$.

2.5. Computational methodology

Interactions between styrene, water and catechol were investigated by performing density functional theory (DFT) based first-principle calculations as implemented in the Vienna *ab initio* simulation package (VASP) [18]. In addition to catechol, other similar structures such as phenol and benzene rings were analyzed in the styrene-water system. The Perdew-Burke-Ernzerhof (PBE) form of electron exchange and correlation and Becke-Johnson form of the van der Waals correction were adopted [19,20]. For total energy calculations, all structures obtained from AIMD are fully relaxed until pressures in x, y, z directions are less than |1| kB. In the unit cell, the total force was reduced to a value less than 10^{-4} eV/Å. For plane-wave basis set, kinetic energy cutoff was taken as 500 eV for all the calculations. The total energy difference between the sequential steps as a convergence criterion for ionic relaxations was set as 10^{-5} eV. In addition, the binding energies of structures, E_B, were calculated using the formula;

$$E_B = (E_{\text{Molecule}} + E_{\text{Double Styrene}} + E_{\text{Double Water}}) - E_{\text{System}} \quad (3)$$

where E_{Molecule} stands for the energy of an isolated molecule e.g. catechol, phenol or benzene, $E_{\text{Double Styrene}}$ is the energy of the total system of two styrene, $E_{\text{Double Water}}$ is the energy of two water molecule and E_{System} is the calculated total energy of the system that includes styrene, water molecule and the molecule. In addition, we performed ab-initio molecular dynamic (AIMD) simulations to understand the interaction of styrene with catechol, phenol, and benzene both in non-aqueous and aqueous media. For the AIMD simulations, NVE ensemble is used. K-point sampling of the Brillouin zone is taken as 1 × 1 × 1 since the periodicity is not required. The temperature is increased from 50 to 450 K up to 10 ps with a time step of 1 fs.

3. Results and discussion

3.1. Characterization of PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$

The synthesized DOPA, tyrosine or phenylalanine conjugated PEG polymers (10 kDa) were first characterized by ^{1}H NMR and UV–Vis spectroscopic techniques. The amine groups of the used amino acids

were protected with Boc groups before PEG conjugation. Fig. 2 shows ^{1}H NMR spectra of functionalized four armed PEG polymers and the precursor sample of PEG-$(NH_2)_4$ in deuterated DMSO. For the ^{1}H NMR spectra of PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$, the presence of signals around 7.0 ppm is the evidence for binding of aromatic groups to PEG-$(NH_2)_4$. In addition, the signal at 1.40 ppm appeared due to the protons on the methyl groups of Boc upon binding of N-Boc-L-DOPA, N-Boc-L-Tyrosine or N-Boc-L-Phenylalanine to PEG-$(NH_2)_4$. The ratio of areas under the signals belong to protons on the methylene groups of PEG (3.62–3.39 ppm) and methyl groups of Boc (1.41 ppm) are also correlated with the ratio of their number of protons which is 896:36. This correlation shows the fully binding of N-Boc-L-DOPA, N-Boc-L-Tyrosine and N-Boc-L-Phenylalanine to the PEG-$(NH_2)_4$ polymers.

Bindings of N-Boc-L-DOPA, N-Boc-L-Tyrosine or N-Boc-L-Phenylalanine to PEG-$(NH_2)_4$ were also confirmed using UV–Vis spectroscopic technique. Fig. 3 (A) shows UV–Vis absorption spectra of 1.94×10^{-4} M Boc-protected amino acids before the PEG conjugation in MES buffer at pH 3.0 to avoid DOPA oxidation [21]. The UV–Vis absorption signals of DOPA, tyrosine and phenylalanine were attributed to the π-π transition of the aromatic ring [22]. N-Boc-L-DOPA has a sharp and strong absorption signal at 279 nm, with a found molar extinction coefficient $\varepsilon_{279} = 2242\ M^{-1}\ cm^{-1}$. UV–Vis spectrum of N-Boc-L-Tyrosine gave a weaker signal at 275 nm with an $\varepsilon_{275} = 1340\ M^{-1}\ cm^{-1}$ compared to the absorption signal of DOPA. The found molar extinction coefficient of N-Boc-L-Phenylalanine, $\varepsilon_{257} = 170\ M^{-1}\ cm^{-1}$, is much smaller than that of DOPA and tyrosine. Its spectrum has the fine structure, wiggles, between 240 and 270 nm with a maximum at 257 nm.

Binding of N-Boc-L-DOPA, N-Boc-L-Tyrosine or N-Boc-L-Phenylalanine to PEG-$(NH_2)_4$ yielded very similar UV–Vis absorption signals with that of free amino acids between 240 and 300 nm (Fig. 3 (B)). As expected, the precursor polymer PEG-$(NH_2)_4$ displays no detectable

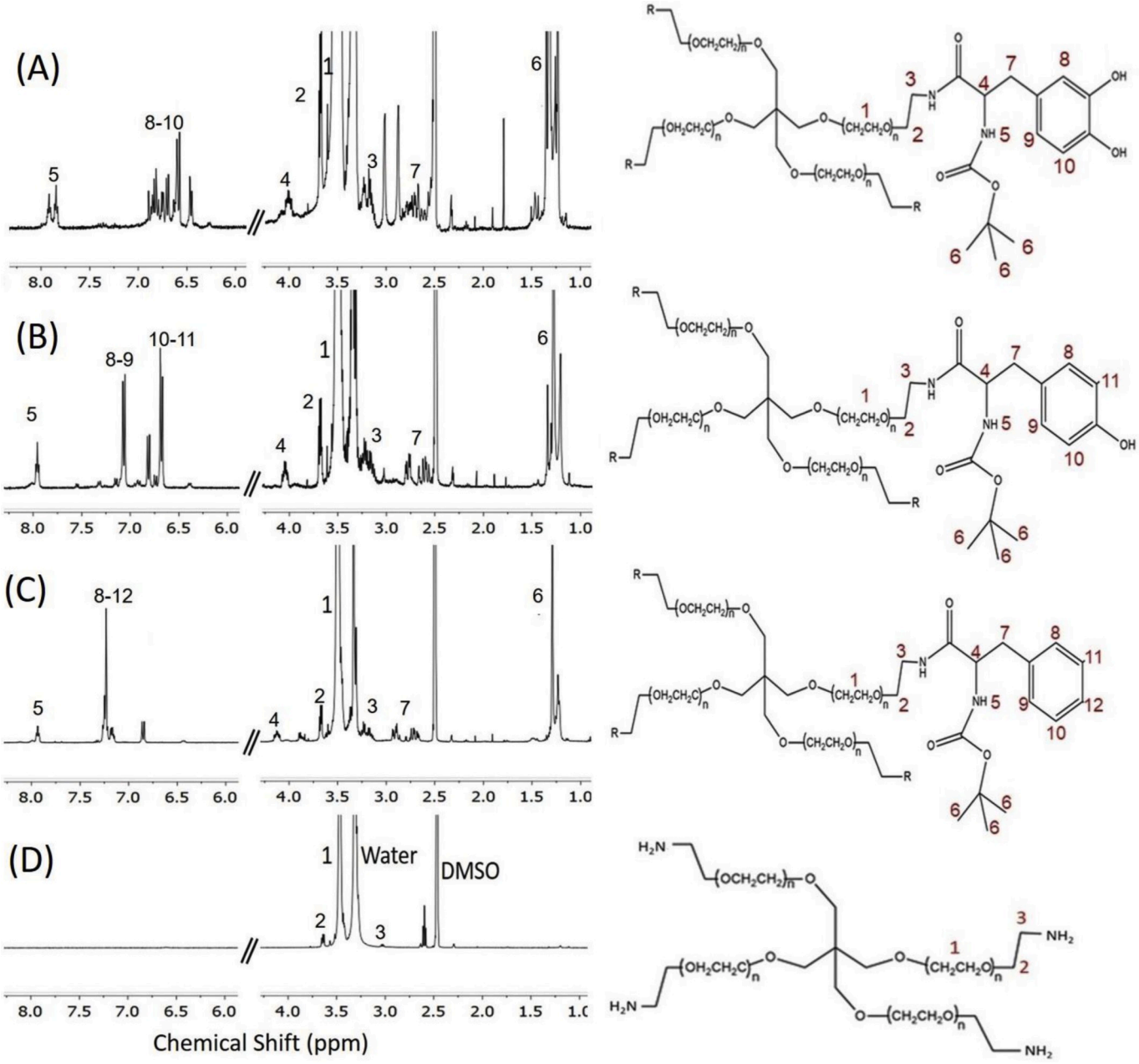

Fig. 2. ^{1}H NMR results and structures of (A) PEG-(N-Boc-L-DOPA)$_4$, (B) PEG-(N-Boc-L-Tyrosine)$_4$, (C) PEG-(N-Boc-L-Phenylalanine)$_4$ and (D) PEG-$(NH_2)_4$ in deuterated DMSO.

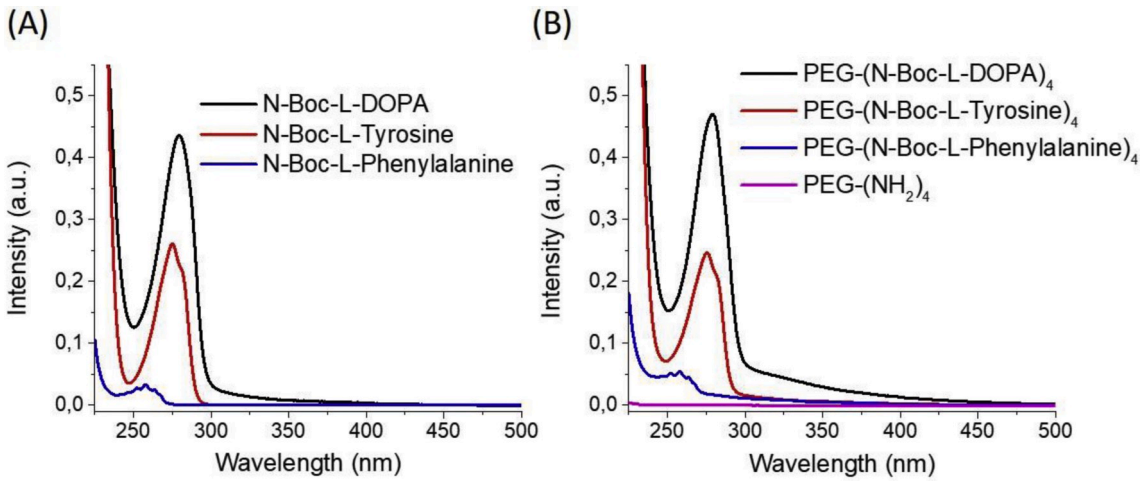

Fig. 3. UV–Vis spectra of 1.94×10^{-4} M N-Boc-L-DOPA, N-Boc-L-Tyrosine and N-Boc-L-Phenylalanine before (A) and after (B) conjugation with 0.485×10^{-4} M PEG-$(NH_2)_4$. UV–Vis spectrum of the precursor PEG-$(NH_2)_4$ was also shown in (B). Samples were dissolved in 0.2 M MES buffer at pH 3.0.

absorption signal. The 0.485×10^{-4} M PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$ have absorption signals at 279, 275 and 257 nm, respectively in MES buffer at pH 3.0. In addition, absorption intensities of amino acids before and after conjugation with PEG are comparable. This means that four armed PEG polymers were conjugated fully with these amino acids.

3.2. Determination of surface adhesion by EPR spectroscopy

Spontaneous wet adhesion properties of DOPA, tyrosine and phenylalanine to colloidal polystyrene (PS) surfaces were studied using EPR spectroscopy. Spin labeling of PS surface with 4-carboxy Tempo allows monitoring the surface of PS nanobeads in aqueous environment. EPR line shapes are very sensitive to the rotational dynamics of spin labels in solution. At room temperature, free spin labels have sharp three-line signals with a rotational correlation time $\tau_R = 20$ ps because of having freely tumbling motion (Fig. S1). However, binding of spin labels to the PS surface restricts the rotational motion of spin labels and increases the rotational correlation time to $\tau_R = 1.6$ ns (Fig. S1). Moreover, it is expected that covering the surface of spin labeled PS (SL-PS) nanobeads with adhesives slows down the rotational motion of spin labels and changes the EPR spectrum.

To gain detailed insight into the adhesion of DOPA to PS surface, we studied with similar amino acids including tyrosine and phenylalanine. These are aromatic amino acids each containing a benzene ring side chain. Additionally, tyrosine and DOPA have one and two hydroxyl groups, respectively (Fig. 1). So, the effect of hydroxyl groups on the wet adhesion of DOPA could be studied with EPR spectroscopy. Samples were dissolved in 0.2 M MES buffer at pH 3.0 to avoid DOPA oxidation [21]. Addition of PEG-(N-Boc-L-Phenylalanine)$_4$ with a final concentration of 90 mg/mL to SL-PS solution did not change the EPR spectrum of the bare SL-PS (Fig. 4 (A)). This showed that PEG-(N-Boc-L-Phenylalanine)$_4$ cannot adhere to PS surface in aqueous solution without applying any force. Therefore, spin labels on PS are not affected from the polymer addition. On the other hand, a second type of spectrum was observed upon addition of PEG-(N-Boc-L-Tyrosine)$_4$ or PEG-(N-Boc-L-DOPA)$_4$ to the SL-PS suspension (Fig. 4 (B) and (C)). This new spectrum with a longer rotational correlation time, $\tau_R = 10$ ns, originated from the covered spin labels on the PS (Fig. 5, S2) beside the uncovered spin labels, $\tau_R = 1.6$ ns (Figs. 5 and S1). Rotational motions of SL on the PS surface become more restricted when tyrosine or DOPA functionalized PEG polymers adhere to the PS surface. We also tested the adhesive role of the precursor PEG-$(NH_2)_4$ polymer. After addition of PEG-$(NH_2)_4$ with a final concentration of 90 mg/mL to SL-PS solution did not change the EPR spectrum which shows no detectable adhesion (Fig. S2).

In addition, EPR spectroscopy allows finding out the percentages of covered and uncovered spin labels on the PS surface. Therefore, the surface coverage could be determined upon addition of adhesive polymers. Simulations of two types spectra belong to uncovered (S1) and covered (S2) SL on PS were used to determine the surface coverage. The sum of their spectra with an appropriate ratio yields the experimental result (Equation (1)). Fig. 5 shows the simulated spectra of SL-PS after

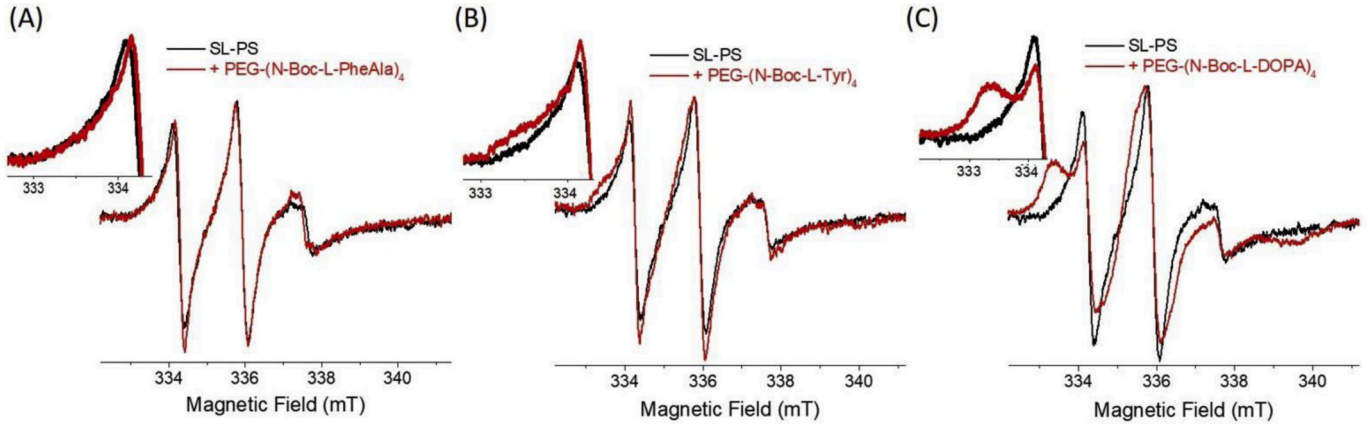

Fig. 4. EPR spectra of SL-PS before (black) and after addition of (A) PEG-(N-Boc-L-Phenylalanine)$_4$, (B) PEG-(N-Boc-L-Tyrosine)$_4$ and (C) PEG-(N-Boc-L-DOPA)$_4$ (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

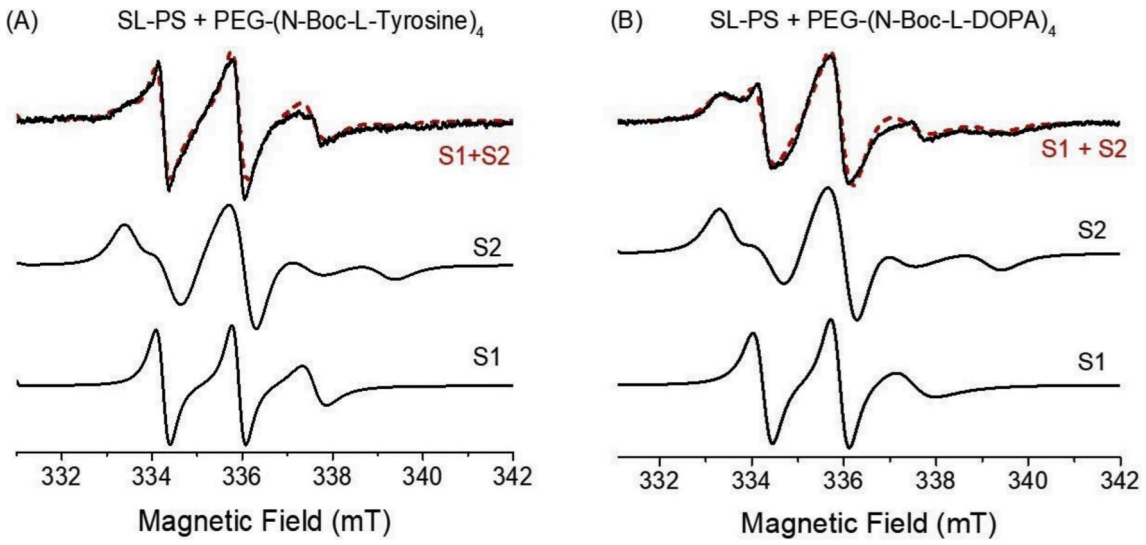

Fig. 5. Simulations of EPR spectra of uncovered (S1) and covered (S2) spin labels on PS with addition of (A) PEG-(N-Boc-L-Tyrosine)$_4$ or (B) PEG-(N-Boc-L-DOPA)$_4$. The sum of appropriate proportions of S1 and S2 yielded the experimental results obtained after addition of PEG-(N-Boc-L-Tyrosine)$_4$ or PEG-(N-Boc-L-DOPA)$_4$ with a final concentration 90 mg/mL (red, dashed lines). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

addition of PEG-(N-Boc-L-Tyrosine)$_4$ or PEG-(N-Boc-L-DOPA)$_4$. Areas under the EPR spectra of uncovered and covered SL on the PS were used to calculate the surface coverage (Equation (2)). Accordingly, after addition of PEG-(N-Boc-L-Tyrosine)$_4$ or PEG-(N-Boc-L-DOPA)$_4$ to SL-PS, the percentages of covered SL on PS were found 50 and 70%, respectively. These findings showed that DOPA functionalized PEG polymer compared to the tyrosine functionalized polymer adhere to the PS surface more extensively.

Here, the experimental EPR studies showed that the adhesion of four armed functionalized PEG polymers to PS increases in the order: PEG-(N-Boc-L-DOPA)$_4$ > PEG-(N-Boc-L-Tyrosine)$_4$, and PEG-(N-Boc-L-Phenylalanine)$_4$ could not adhere to PS surface in the aqueous condition. Therefore, interactions between DOPA and styrene rings are larger than the interactions between tyrosine and styrene rings in the presence of water. On the other hand, the interactions between phenylalanine and styrene rings must be very weak to achieve the wet adhesion.

3.3. Binding energies and AIMD simulations of the styrene systems

In order to understand the reason of spontaneous wet adhesive ability of DOPA and tyrosine but not phenylalanine functionalized PEG polymers to PS surface, total energy and molecular dynamics calculations were performed. Instead of DOPA, tyrosine and phenylalanine amino acids, the interactive sides of them such as catechol, phenol and benzene molecules, respectively, were used in the DFT calculations.

Although, the DOPA based adhesions on hydrophobic surfaces e.g. PS and CH_3-terminated monolayers, have been studied experimentally [2,8,10], only one theoretical study was performed up to now [23]. Levine et al. applied a combination of surface forces apparatus (SFA) and molecular dynamics simulations on a DOPA-containing peptide which adheres strongly to CH_3-terminated monolayers but adheres weakly to OH-terminated monolayers [23]. In the literature, the most DOPA based adhesive both experimental and/or theoretical studies have been investigated on hydrophilic and metal surfaces, e.g. silica, TiO_2, mica, Cu (100), etc. [8,24–28].

While PS surface is considered hydrophobic, the benzene groups on the PS show hydrogen-bond accepting character. The estimated hydrogen bond strength between the aromatic ring of PS and water is higher than 110 meV which indicates the hydrophilic character of the aromatic moiety [29]. Therefore, spontaneous adsorption of amino acids functionalized PEG polymers to PS surfaces could be affected by the pre-adsorbed water molecules on PS.

In Fig. 6 (A), AIMD simulations revealed that two water molecules interact with two styrene molecules with a binding energy 485 meV due to the established hydrogen bonds between water-water and water-styrene, and also aromatic interactions between styrenes. After 2 ps, water molecules diffused between two styrene rings causing them tilting out (Fig. 6 (A), 2 ps, side view). Addition of a catechol molecule to that system created new hydrogen bonds between two neighboring hydroxyl groups of catechol and water molecules. Furthermore, the conformation of styrene rings which were already tilted out enhanced the interaction between the catechol and styrene rings via π-π stacking in the presence of water (Fig. 6 (B), 2 ps, side view). Therefore, the total binding energy of the catechol included system increased to 761 meV. Similar to the case of a catechol addition, a phenol molecule addition increased the total binding energy of system to 733 meV. Since phenol molecule has only one hydroxyl group, it forms less hydrogen bonds with water molecules compared to the number of hydrogen bonds formed between catechol and water. Also, AIMD simulations showed that styrene rings which were tilted out in water increased the phenol-styrene ring interactions via π-π stacking, too (Fig. 6 (C), 2 ps, side view). On the other hand, addition of a benzene molecule to the system increased the total binding energy of system only to 562 meV from 485 meV. Since benzene does not have any hydroxyl groups, interaction between benzene and water is weaker than the interactions between catechol-water or tyrosine-water. In addition, AIMD simulations showed that styrene rings have a relatively closed structure (Fig. 6 (D), 2 ps, side view) upon addition of a benzene molecule compared to the open structure of styrenes observed upon addition of catechol/phenol in water (Fig. 6 (B, C), 2 ps, side views) which might be the reason of getting a less total binding energy.

Without water systems, the conformations of styrene rings with respect to each other are also crucial for catechol, phenol and benzene binding. Fig. 7 shows the AIMD simulations of catechol-double styrene, phenol-double styrene and benzene-double styrene with total binding energies 90 meV, 260 meV and 120 meV, respectively. Obtaining the lowest total binding energy (90 meV) in the system of catechol-double styrene could be explained due to the higher hydrophilicity of catechol compared to the others. On the other hand, the benzene ring with higher hydrophobicity showed only a slightly better binding property (120 meV) to the styrene rings. Much higher binding energy was observed for the phenol-double styrene system (260 meV) than the other

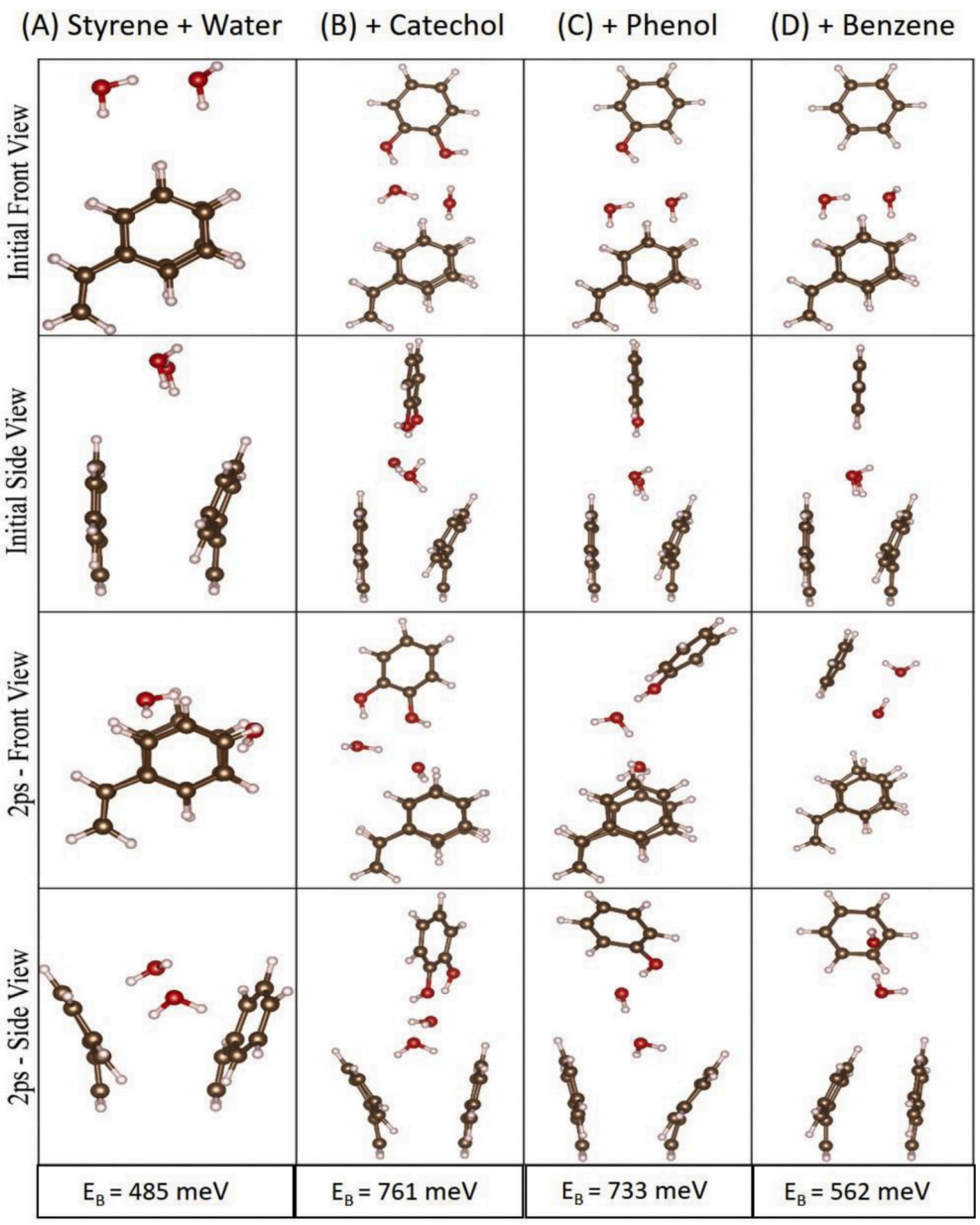

Fig. 6. AIMD simulations of the water including systems: initial and after 2 ps configurations with front and side views for the different systems composed of (A) two styrene and two water molecules, and after addition of (B) one catechol molecule, (C) one phenol molecule and (D) one benzene molecule. Total binding energies of the systems are shown under the views. Red, white and brown atoms represent oxygen, hydrogen and carbon atoms, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

two systems. In the absence of water, externally tilted styrene structure which induced the binding was observed only after addition of phenol. This showed that both hydroxyl group and benzene ring collaborate together to bind to styrene rings in the absence of water.

The combined results of EPR measurements and DFT calculations provide the importance of hydroxyl groups on the wet adhesion of DOPA/tyrosine to PS surface. Semoto et al. have showed the role of hydroxyl groups and adsorbed water molecules in the adhesion interface between a fragment of epoxy resin and a water adsorbed aluminum oxide surface by using DFT calculations [30]. The results demonstrated that the hydrogen-bond network via adsorbed water molecules significantly affects the adhesion mechanism. Here, in water, externally tilted styrene conformation enhances the interaction between DOPA/tyrosine with styrene rings. Hydroxyl groups on the DOPA and tyrosine interact with water molecules via hydrogen bonding, and move together which enhances the adhesion on PS. However, phenylalanine without hydroxyl groups could not interact with water. So, in the presence of water, pre-adsorbed water molecules on the styrene surface do not allow phenylalanine to be close to the styrene surface. This is also supported with the AIMD simulations of the systems in the absence of water. Without water, styrene rings externally tilted with respect to each other only with phenol addition which increases the binding. Hydrophilic and hydrophobic interactions together determine the binding of catechol to the styrene surface.

4. Conclusion

In the literature, the role of DOPA hydroxyl groups in the wet adhesion to the hydrophilic surfaces e.g. silica, mica and glass was investigated in detail. However, the role of DOPA hydroxyl groups in the wet adhesion to the hydrophobic surface was not studied before. Here, we experimentally and theoretically investigated the effects of hydroxyl groups on the wet adhesion to the hydrophobic polystyrene (PS) surface. Three amino acids with similar structures except the number of their hydroxyl groups, DOPA, tyrosine and phenylalanine, were attached to the four chain ends of a PEG polymer; PEG-(N-Boc-L-DOPA)$_4$, PEG-(N-Boc-L-Tyrosine)$_4$ and PEG-(N-Boc-L-Phenylalanine)$_4$.

Their wet adhesive properties were compared by using EPR

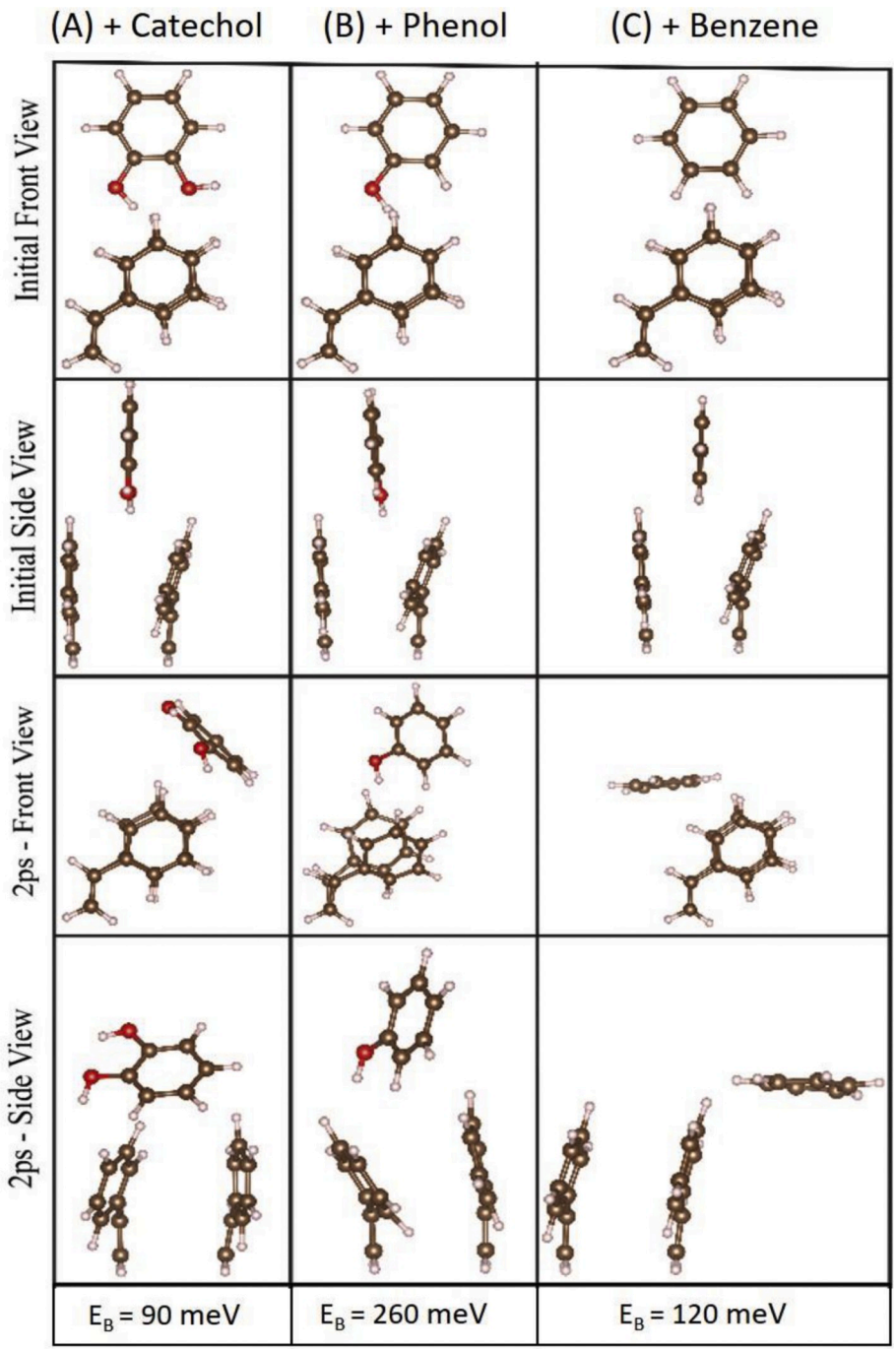

Fig. 7. AIMD simulations of the systems without water: initial and after 2 ps configurations with front and side views for the different systems composed of (A) two styrene and one catechol, (B) two styrene and one phenol and (C) two styrene and one benzene. Total binding energies of the systems are shown under the views. Red, white and brown atoms represent oxygen, hydrogen and carbon atoms, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

spectroscopy in terms of their surface coverages. Spin labeled polystyrene (SL-PS) was prepared in water to be used as a model surface for the EPR measurements. Without applying force, PEG-(N-Boc-L-DOPA)$_4$ and PEG-(N-Boc-L-Tyrosine)$_4$ were able to adhere to SL-PS surfaces with the percentages of surface coverages 70% and 50%, respectively. However, PEG-(N-Boc-L-Phenylalanine)$_4$ did not adhere to the SL-PS surface noticeably. This showed that hydroxyl groups on the benzene rings enhance the adhesion to PS in water. In addition, the spontaneous wet adhesion was not achieved by phenylalanine without having a hydroxyl group on the benzene side. To further confirm the hydroxyl group effects on the wet adhesion, DFT calculations were applied to the systems including two water molecules and double styrene rings. For the DFT calculations, catechol, phenol and benzene molecules were used instead of DOPA, tyrosine and phenylalanine, respectively. After addition each of these molecules to the double styrene - double water systems, the system binding energies increases in the order of benzene < phenol < catechol with energies 562 meV, 733 meV and 762 meV. This result is in parallel with the result obtained from the EPR measurements that showed the similar but better adhesion of DOPA than tyrosine, and also not a significant adhesion of phenylalanine to the PS surface. In water, the better adhesions of DOPA and tyrosine than phenylalanine to PS surface could be due to the formed hydrogen bonds between DOPA/tyrosine and water molecules. Pre-adsorbed water molecules on styrene induce DOPA/tyrosine to be more interactive with styrene rings through π-π stacking. However, phenylalanine without hydroxyl group does not interact with water on the styrene surface and cannot be close enough to achieve strong bindings with styrene. In addition, in the absence of water, DFT calculations showed that finding one hydroxyl group on the

phenol enhances the interaction between phenol and styrenes more than the interactions between catechol-styrenes and benzene-styrenes in the order of catechol < benzene < phenol.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.matchemphys.2019.122606.

References

[1] P.K. Forooshani, B.P. Lee, Recent approaches in designing bioadhesive materials inspired by mussel adhesive protein, J. Polym. Sci., Part A: Polym. Chem. 55 (2017) 9–33.
[2] C. Leng, Y. Liu, C. Jenkins, H. Meredith, J.J. Wilker, Z. Chen, Interfacial structure of a DOPA- inspired adhesive polymer studied by sum frequency generation vibrational spectroscopy, Langmuir 29 (2013) 6659–6664.
[3] J.H. Waite, Mussel adhesion – essential footwork, J. Exp. Biol. 220 (2017) 517–530.
[4] B.P. Lee, P.B. Messersmith, J.N. Israelachvili, J.H. Waite, Mussel-inspired adhesives and coatings, Annu. Rev. Mater. Res. 41 (2011) 99–132.
[5] B. Yang, C. Lim, D.S. Hwang, H.J. Cha, Switch of surface adhesion to cohesion by dopa-Fe^{3+} complexation, in response to microenvironment at the mussel plaque/substrate interface, Chem. Mater. 28 (2016) 7982–7989.
[6] Z. Gao, L. Duan, Y. Yang, W. Hu, G. Gao, Mussel-inspired tough hydrogels with self repairing and tissue adhesion, Appl. Surf. Sci. 427 (2018) 74–82.
[7] W. Zhang, H. Yang, F. Liu, T. Chen, G. Hu, D. Guo, Q. Hou, X. Wu, Y. Su, J. Wang, Molecular interactions between DOPA and surfaces with different functional groups: a chemical force microscopy study, RSC Adv. 7 (2017) 32518–32527.
[8] Q. Lu, E. Danner, J.H. Waite, J.N. Israelachvili, H. Zeng, D.S. Hwang, Adhesion of mussel foot proteins to different substrate surfaces, J. R. Soc. Interface 10 (2013) 20120759.
[9] J. Yu, Y. Kan, M. Rapp, E. Danner, W. Wei, S. Das, D.R. Miller, Y. Chen, J.H. Waite, J.N. Israelachvili, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 15680–15685.
[10] Y. Akdogan, W. Wei, K.Y. Huang, Y. Kageyama, E.W. Danner, D.R. Miller, N.R. M. Rodriguez, J.H. Waite, S. Han, Intrinsic surface-drying properties of bioadhesive proteins, Angew. Chem. Int. Ed. 53 (2014) 11253–11256.
[11] İ. Kırpat, Y. Göksel, E. Karakuş, M. Emrullahoğlu, Y. Akdogan, Determination of force-free wet adhesion of mussel-inspired polymers to spin labeled surface, Mater. Lett. 205 (2017) 48–51.
[12] Y. Göksel, Y. Akdogan, Increasing spontaneous wet adhesion of DOPA with gelation characterized by EPR spectroscopy, Mater. Chem. Phys. 228 (2019) 124–130.
[13] C. Altenbach, C.J. Lopez, K. Hideg, W.L. Hubbell, Exploring structure, dynamics, and topology of nitroxide spin-labeled proteins using continuous-wave electron paramagnetic resonance spectroscopy, Methods Enzymol. 564 (2015) 59–100.
[14] Y. Akdogan, M. Emrullahoglu, D. Tatlıdil, M. Ucuncu, G. Cakan-Akdogan, EPR studies of intermolecular interactions and competitive binding of drugs in a drug–BSA binding model, Phys. Chem. Chem. Phys. 18 (2016) 22531–22539.
[15] G. Giorgioni, F. Claudi, S. Ruggieri, M. Ricciutelli, G.F. Palmieri, A. Di Stefano, P. Sozio, L.S. Cerasa, A. Chiavaroli, C. Ferrante, G. Orlando, R.A. Glennon, Design, synthesis, and preliminary pharmacological evaluation of new imidazolinones as LDOPA prodrugs, Bioorg. Med. Chem. 18 (2010) 1834–1843.
[16] B.P. Lee, J.L. Dalsin, P.B. Messersmith, Synthesis and gelation of DOPA-modified poly(ethylene glycol) hydrogels, Biomacromolecules 3 (2002) 1038–1047.
[17] S. Stoll, A. Schweiger, EasySpin, a comprehensive software package for spectral simulation and analysis in EPR, J. Magn. Reson. 178 (2006) 42–55.
[18] G. Kresse, J. Furthmüller, Efficient iterative schemes for *ab initio* total-energy calculations using a plane-wave basis set, Phys. Rev. B 54 (1996) 11169.
[19] J.P. Perdew, K. Burke, M. Ernzerhof, Generalized gradient approximation made simple, Phys. Rev. Lett. 77 (1996) 3865.
[20] S. Grimme, S. Ehrlich, L. Goerigk, Effect of the damping function in dispersion corrected density functional theory, J. Comput. Chem. 32 (2011) 1456–1465.
[21] M. Cencer, Y. Liu, A. Winter, M. Murley, H. Meng, B.P. Lee, Effect of pH on the rate of curing and bioadhesive properties of dopamine functionalized poly(ethylene glycol) hydrogels, Biomacromolecules 15 (2014) 2861–2869.
[22] J.M. Antosiewicz, D. Shugar, UV–Vis spectroscopy of tyrosine side-groups in studies of protein structure. Part 2: selected applications, Biophys. Rev. 8 (2016) 163–177.
[23] Z.A. Levine, M.V. Rapp, W. Wei, R.G. Mullen, C. Wu, G.H. Zerze, J. Mittal, J. H. Waite, J.N. Israelachvili, J.-E. Shea, Surface force measurements and simulations of mussel-derived peptide adhesives on wet organic surfaces, Proc. Natl. Acad. Sci. U.S.A. 113 (2016) 4332–4337.
[24] S.A. Mian, L.C. Saha, J. Jang, L. Wang, X. Gao, S. Nagase, Density functional theory study of catechol adhesion on silica surfaces, J. Phys. Chem. C 114 (2010) 20793–20800.
[25] S.A. Mian, Y. Khan, M. Ajmal, M.A. Khan, S. Ahmad, Investigation of the adhesion mechanism of Marine Mussel's foot protein: adsorption of L-dopa on silica α- & β-cristobalite using density functional theory (DFT), Mater. Today Proc. 4 (2017) 4912–4918.
[26] U. Terranova, D.R. Bowler, Adsorption of catechol on TiO(2) rutile (100): a density functional theory investigation, J. Phys. Chem. C 114 (2010) 6491–6495.
[27] Q. Lin, D. Gourdon, C. Sun, N. Holten-Andersen, T.H. Anderson, J.H. Waite, J. N. Israelachvili, Adhesion mechanisms of the mussel foot proteins mfp-1 and mfp-3, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 3782–3786.
[28] S.-K. Chen, B.-C. Wang, T.-G. Zhou, W.-Z. Huang, Theoretical study of the adsorption of DOPA-quinone and DOPA-quinone chlorides on Cu (100) surface, Appl. Surf. Sci. 257 (2011) 7938–7943.
[29] O.N. Tretinnikov, Hydrophilic (Hydrogen-Bonding) polystyrene surface by substrate-induced surface segregation of benzene groups, Langmuir 16 (2000) 2751–2755.
[30] T. Semoto, Y. Tsuji, K. Yoshizawa, Molecular understanding of the adhesive force between a metal oxide surface and an epoxy resin: effects of surface water, Bull. Chem. Soc. Jpn. 85 (2012) 672–678.