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Polydopamine as a Catalyst for Thiol Coupling

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In biological systems, disulfide bonds are formed efficiently under mild conditions without the release of harmful byproducts. Inspired by nature, we report a biomimetic polydopamine (PDA) catalyst for oxidative thiol coupling. This reaction was accelerated with only a small amount of PDA particles in neutral, weakly alkaline, and even weakly acidic aqueous media at room temperature under an air atmosphere. The catalytic particles were facilely separated and were reused without a decrease in activity. The entire process is totally biofriendly, including the synthesis of the PDA particles. This route is especially useful for the synthesis of pharmaceutical molecules.

It is well known that disulfides are very important chemicals that widely exist in biological systems.[1–6] They play a crucial role in maintaining the conformational stability and biological activity of proteins[1,2] and participate in the adhesion process of mussel foot protein.[4,5] Over the past decades, these chemicals have received much attention as a result of their artificial applications in synthesizing pharmaceutical molecules,[7,8] constructing functional materials,[9–11] building dynamic combinatorial libraries,[12,13] and preparing sulfur-containing organic compounds.[14,15] Numerous methods have been developed to form disulfide bonds in both scientific laboratories and industrial factories. Up to now, the most straightforward approach is the oxidative coupling of thiols.[1,6,16–22] In organic media, thiols are oxidized into disulfides by oxygen from the air, catalyzed by heavy metal ions,[1,16] metal nanoparticles,[17] or other organic catalysts.[18,19] Furthermore, this coupling has also been achieved by the redox reaction of thiols with oxidants such as iodine[20] and graphene oxide.[21] In aqueous media, aerobic oxidative coupling can be easily conducted in alkaline solution.[22] Nevertheless, this method is not suitable to thiols that are unstable in alkaline media, for instance, basic fibroblast growth factor.[6] Other approaches have been developed in neutral or acidic media. Typical examples include coupling thiols by using organic oxidants[6] and metal-ion-catalyzed oxidation in the presence of oxygen.[9,22] However, these methods suffer from difficulties in the removal of harmful impurities and reuse of the catalysts, which may limit their suitability, especially in pharmaceutical applications.

Disulfides are formed in nature in an efficient and controllable way under mild conditions. For example, disulfide bonds are accurately generated for protein folding through an enzyme-catalyzed approach in living cells. The two thiol groups of the enzyme DsbB are oxidized into a disulfide bond by ubiquinone in *E. coli*. Subsequently, ubiquinone is regenerated by aerobic oxidation, whereas the DsbB enzyme transfers the disulfide bond to the DsbA enzyme. Finally, DsbA transfers its disulfide bond for protein folding in bacterial cells.[23,24] Another example is that thiols are efficiently oxidized into disulfides through a redox process with dopamine quinone motifs in mussel foot proteins.[4] In this case, the dopamine quinone motifs are reduced to control the oxidative cross-linking reaction of the mussel foot proteins. This redox process and the resulting cross-linked polydopamine (PDA) play important roles for the unique adhesion of mussels on various surfaces.[4] Lee et al. inspired this mechanism[25] and opened the door to the simple and versatile modification of material surfaces through the oxidative formation of PDA.[26–33] Although there are still challenges to clarify the chemical structure of synthetic PDA, it is generally agreed that dopamine must be oxidized into dopamine quinone and that there are abundant quinone motifs in the corresponding polymer.[34–36] These mechanisms have been summarized by d'Ischia et al.[37] Therefore, it is reasonable for us to develop a biomimetic route for the synthesis of disulfides by using the quinone motifs in PDA.

We herein report a PDA-based heterogeneous catalyst for the synthesis of disulfides by aqueous thiol coupling. Our method is totally biofriendly and no waste or harmful chemicals are produced except for water. The PDA catalyst particles can be easily separated from the reaction media and reused with no marked decrease in catalytic activity. Besides, our catalyst can be used in weakly alkaline, neutral, and even weakly acidic aqueous media at room temperature.

It is well known that dopamine self-polymerizes into PDA particles in weakly alkaline aqueous media, oxidized by oxygen dissolved in solution. PDA particles were synthesized in Tris buffer of pH 8.5 in air. SEM and TEM images indicate that the particles have diameters ranging from 100 to 500 nm and form aggregates (Figures S1a and S2 in the Supporting Information). However, these particles can be dispersed well in phosphate-buffered solution and can be completely removed from the solution by passing through a 0.22 μm cellulose acetate membrane (Figure S3).

The thiol coupling reactions were catalyzed by PDA particles at room temperature in air. Various thiols (0.15 mmol) were studied in phosphate-buffered D_2O solutions (0.2 M) at pH 6.0, 7.0 and 8.0. This medium is convenient to identify the coupling

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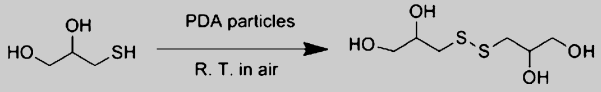
product and the conversion. It is reasonable to regard that the degree of ionization of the thiols is similar to that in corresponding phosphate-buffered H_2O solutions. For example, 1-mercaptoglycerol (MGL) was coupled to form bis(2,3-dihydroxypropyl)disulfide by the catalysis of PDA particles. The synthesized bis(2,3-dihydroxypropyl)disulfide was isolated by liquid-phase chromatography and was identified by using ^{1}H NMR and FTIR spectroscopy and MS (Figures S5 and S6) to confirm that only the thiol-coupling reaction occurred. The results show that the coupling conversion is significantly increased by adding only a small amount of PDA particles with a reaction time of 24 h (Table 1, entry 1 vs. 2). Besides, our results also indicate that the coupling time can be decreased from 24 to 8 h simply by increasing the amount of PDA particles (Table 1, entries 1, 3, 4). Thus, the reaction rate can be easily tuned by the dose of PDA particles.

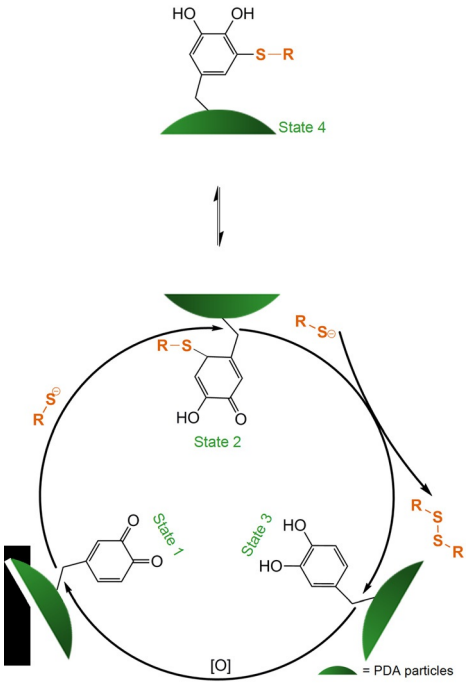
Table 1. Coupling reaction of 0.15 mM 1-mercaptoglycerol in 1 mL buffered D_2O solution under various conditions.



Entry	Catalyst [mg]	pH	Reaction time [h]	Conversion[a] [%]
1	4.5	≈7.0	24	96
2	0	≈7.0	24	<5
3	9.0	≈7.0	12	94
4	18.0	≈7.0	8	94
5	4.5	≈6.0	48	63
6	0	≈6.0	48	15
7	4.5	≈8.0	12	97
8	0	≈8.0	12	5.9
9	13.5	≈6.0	24	90
10	4.5[b]	≈7.0	24	trace

[a] Conversion was determined by NMR spectroscopy. [b] Dopamine (4.5 mg) was used as the catalyst, under the same conditions as those listed in entry 1.

We suggest that the coupling reaction of thiols catalyzed by PDA should follow a mechanism similar to that of mussel adhesion[4,5] and that of disulfide bond formation in DsbB[23,24] in nature. Scheme 1 shows that the proposed mechanism consists of three steps: one, nucleophilic attack of the quinone groups on the PDA particles (state 1) by the first thiolate anion to form a thiol–dopamine adduct (state 2), as illustrated by previous work;[38] two, attack of the thioether adduct by a second thiolate anion to form a disulfide and phenol-state dopamine (state 3); three, oxidization of phenol-state dopamine into quinone-state dopamine by oxygen dissolved in the solution. The quinone groups on the PDA particles were identified by X-ray photoelectron spectroscopy (XPS) (Figure S4), which is in accordance with the previous report.[39] To further understand this mechanism, PDA particles were used to sequentially catalyze the individual oxidative couplings of sodium 3-mercapto-1-propanesulfonate (MPS), MGL, and sodium 3-mercapto-1-propanesulfonate (shown in Figure 1). We found that the ζ potential of the initial PDA particles is



Scheme 1. Proposed mechanism of the PDA-catalyzed thiol coupling reaction.

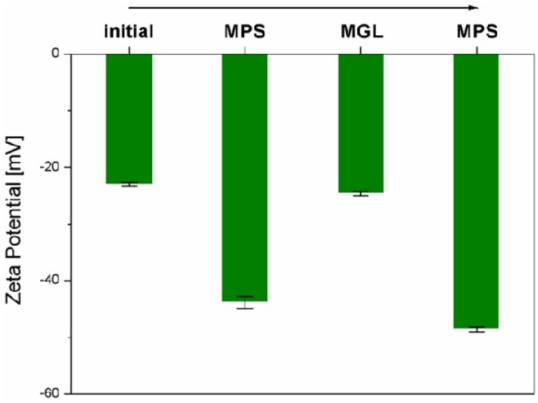


Figure 1. The ζ potential of the PDA particles after sequentially catalyzing the oxidative coupling of MPS, MGL, and MPS.

−23.0 mV. This value decreases to −43.9 mV after catalyzing the coupling of MPS, which indicates that MPS links the PDA particles, shown as state 4 in Scheme 1. However, after catalyzing the coupling of MGL the value increases to −24.6 mV, which is similar to that of the initial PDA particles. The reason is that the MPS motifs tethered on the PDA particles are replaced by MGL groups. In detail, MGL thiolate anions attack the catalyst in state 2, which can be reversibly transformed from state 4 (in Scheme 1) to form disulfides and phenol-state dopamine. Afterwards, other MGL thiolate anions attach to the catalyst during the reaction. The ζ potential of these PDA particles finally decreases to −48.6 mV after catalyzing the coupling of MPS for the second time. This is similar to what occurs to the PDA particles after catalyzing the coupling of MPS for the first time, which suggests that the MGL motifs tethered on

the PDA particles are totally replaced by MPS groups. These results strongly illustrate that the catalytic reaction follows our suggested mechanism. Dopamine is not able to catalyze the coupling reaction according to this mechanism, which is in agreement with our experimental results (Table 1, entry 10). It is also reasonable to consider that the catalysis process mainly happens on the surfaces of the PDA particles. This phenomenon was also observed by Vaish et al.[41]

We studied the influence of pH on the catalysis properties. The reaction can be catalyzed in neutral, weakly alkaline, and weakly acidic aqueous media (Table 1, entries 1, 2, 5–9). However, the rate of the reaction decreases as the pH decreases. This is because the catalyzed coupling needs a thiolate ion as an intermediate,[4,5,23,24] and the concentration of this ion increases in weakly alkaline solution.

We also evaluated the reusability of the PDA particles as a catalyst for the coupling reactions. Typical results are shown in Figure 2 with the formation of bis(2,3-dihydroxypropyl)disulfide as an example. The mass loss of the catalyst after each run

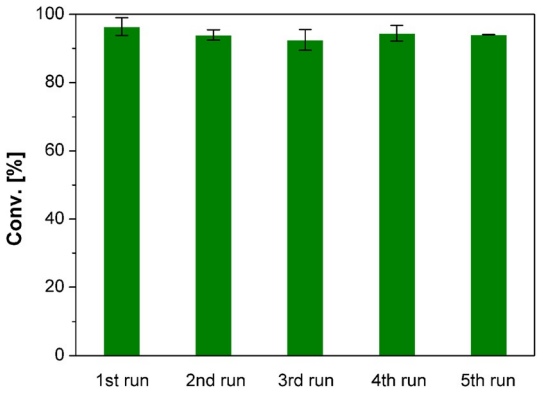


Figure 2. Reusability of the PDA particles as a catalyst for the coupling reaction of thiols. All reactions were performed by using 0.15 mM MGL as the reactant and PDA particles as the catalyst in buffered D_2O solution (pH ≈ 7.0) at room temperature in air for 24 h. After each run, the catalyst was removed by centrifugation, freeze-dried, and weighed. The amount of solution for the next run was determined according to the weight of the catalyst left after the former run. The PDA dose was 4.5 mg for the first run.

is approximately 10% because the PDA particles are easily dispersed in water and some of them are inevitably removed along with water during each washing step. The PDA particles can be reused over at least five runs after separation from the reaction media by centrifugation without any detectable decrease in the catalytic activity. A thiol molecule can attach to the PDA particles to form a C–S bond through a Michael addition reaction[25,38–40] (shown as state 4 in Scheme 1). However, state 4 can reversibly transform into state 2 according to our suggested mechanism. Thus, the thiolated PDA in state 2 still has the ability to catalyze the coupling reaction. Besides, thiols tethered on the PDA particles can finally couple with other thiols in the solution to form disulfides. Notably, in a previous study that used thiols to functionalize polydopamine, the reaction medium was deoxidized to avoid the formation of disulfides.[25] In contrast, in our experiments the reaction medium is exposed to air during the entire reaction, as oxygen is necessary for the regeneration of the PDA catalyst. We suggest that exposure to air and the large surface area of PDA make the thiol coupling reaction the dominant process. During the catalyzed reaction, the morphology of the PDA particles does not undergo any clear change relative to that of the original particles, as observed by SEM (Figure S1 b).

We subjected a variety of commercially available water-soluble thiols to the catalyzed thiol-coupling reaction. We changed the coupling conditions for different thiols to distinguish the reaction rate of the catalyzed reaction and the noncatalyzed one. The PDA particles facilitated the coupling of all the selected thiols, as shown in Figure 3, whereas the reaction rate was

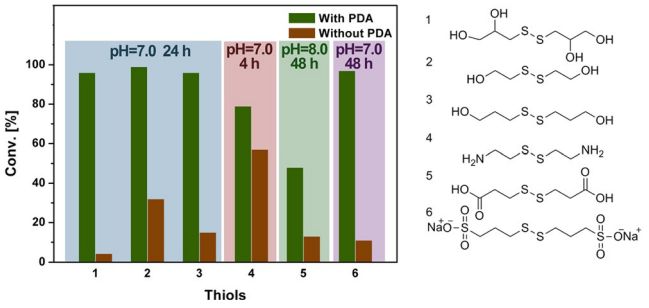


Figure 3. Catalyzed coupling reactions of various thiols. In each reaction, 0.15 mM thiol was used as the reactant and 4.5 mg PDA particles was used as the catalyst. They were incubated in 1 mL buffered D_2O solution at room temperature in air for a certain period of time. The pH value of the solution and the reaction time are shown.

slightly different. Thiols containing hydroxy groups, carboxylic groups, or sulfonate groups showed similar results during the coupling reaction. The slight rate increase for 2-aminoethanethiol can be ascribed to a fast noncatalyzed reaction. The low reaction rate of the coupling of 2-mercaptoacetic acid is the result of the difficulty to form the thiolate ion.

We also tested several commonly used organic solvents to study whether our catalyst was suitable for reaction in organic media. However, the PDA particles might be partially dissolved in polar organic solvents, such as DMSO, DMF, and methanol, as the solution turned brown after the PDA particles were dispersed and then separated from these solvents. Besides, this reaction can also not be performed in nonpolar organic solvents, such as hexane, as the PDA particles cannot be easily dispersed in these solvents. Therefore, we suggest that it is very difficult to find a proper organic solvent in which this reaction can be performed.

In conclusion, we demonstrated the use of a biomimetic PDA catalyst for disulfide synthesis by thiol coupling under mild conditions. The reaction can be efficiently performed in a neutral, weakly alkaline, or weakly acidic environment. We suggest that the catalysis follows a mechanism similar to that of mussel adhesion and disulfide formation in DsbB in nature by using dopamine quinone motifs as the active center. This method shows great potential in preparing disulfides, especial-

ly for pharmaceutical molecules owing to the easy separation of the catalyst and the totally biofriendly chemistry.

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Keywords: biomimetic catalysts · heterogeneous catalysis · polydopamine · sulfur · thiol coupling

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