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Directed Assembly of Carbon Nanotubes at Liquid−Liquid Interfaces: Nanoscale Conveyors for Interfacial Biocatalysis

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We have discovered that single-walled carbon nanotubes (SWNTs) can be directed to aqueous−organic interfaces with the aid of surfactants. This phenomenon can be used to transport adsorbed proteins from a bulk aqueous phase to an interface, thereby enabling the unique properties of nanomaterials to be exploited at an interface. In particular, SWNTs provide a high intrinsic surface area without intraparticle diffusional limitations. As a result, the nanotube-mediated interfacial assembly of enzymes increases the rate of interfacial biotransformations by over 3 orders of magnitude relative to that for native enzyme in the bulk aqueous phase. Furthermore, we demonstrate that this concept can be extended to other nanomaterials, thereby providing a general strategy for performing highly efficient biphasic reactions.

We observed that an aqueous dispersion of purified SWNTs, prepared using our previously reported procedure,[1] when contacted with an equal volume of hexane (or isooctane, $CHCl_3$, or CH_2Cl_2) containing a 2 mM concentration of the anionic surfactant Aerosol-OT (AOT), led to the transfer of SWNTs from the aqueous phase (Figure 1a) to the interface (Figure 1b). Interfacial assembly was also observed when a dispersion of SWNTs in hexane was contacted with solutions of the neutral surfactant Tween, the cationic surfactant dodecyltrimethylammonium bromide (DTAB), or the cationic lipid 1,2-dimyristoyl-3-trimethylammonium propane (DMTAP). On the basis of this finding, we reasoned that the nanotubes may serve as carriers to transport proteins to an aqueous−organic interface; the resulting protein−nanotube assemblies could thus be used to enhance the rate of interfacial transformations.

Biocatalytic transformations of a variety of water-insoluble compounds, including epoxides and steroids, are of considerable commercial interest for the synthesis of pharmaceuticals and fine chemicals.[2] While aqueous−organic systems are advantageous for such biotransformations, biphasic reactions are typically limited by the poor transport of reactants from the organic phase into the aqueous enzyme phase and the loss of enzyme activity, possibly due to denaturation at the interface.[3] The SWNT-mediated assembly of enzymes at the aqueous−organic interface may help overcome these transport limitations. Furthermore, the nanoscale environment of SWNTs can enhance the function and stability of adsorbed proteins to a greater extent than microscale or macroscopic supports.[4] Consequently, the immobilization of proteins on SWNTs may also aid in the retention of near native function of enzymes incorporated at the aqueous−organic interface.

To that end, we adsorbed a well-characterized protein soybean peroxidase (SBP) onto SWNTs in aqueous buffer following our previously reported protocol.[1] This enzyme requires two substrates: a hydrophobic phenol and the hydrophilic H_2O_2. This system is therefore ideally suited for interfacial catalysis. In the presence of AOT, the SWNT−SBP conjugates assembled at the hexane−water interface. No protein or SWNTs were detected in either of the bulk phases as determined by UV−vis spectroscopy. The catalytic activity of the interfacial SWNT−SBP was measured

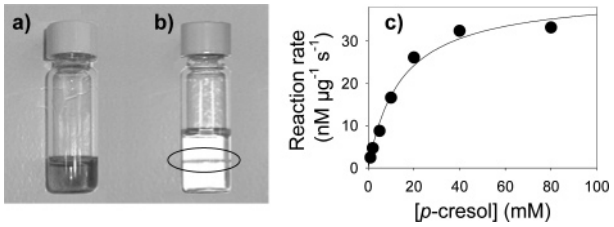

Figure 1. Photographs demonstrating the assembly of SWNTs at the interface: (a) suspension of SWNTs in water and (b) the interfacial assembly of SWNTs on addition of a solution of AOT in hexane (2 mM) to the aqueous dispersion of SWNTs. (c) Effect of substrate concentration on the initial rate of oxidation of *p*-cresol catalyzed by SWNT−SBP at the interface.

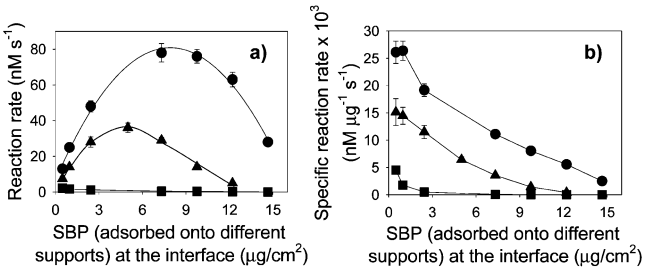

Figure 2. Influence of the concentration of SBP at the interface: (a) absolute reaction rates and (b) specific reaction rates for SBP adsorbed onto SWNTs (●), hydrophobic SiNPs (20 nm) (▲), and hydrophobic nanoporous glass beads (■) at the interface. The error bars indicate the standard deviation of triplicate measurements.

using a model peroxidase substrate, *p*-cresol. The product was detectable only in the organic phase, and the initial reaction rate for interfacial SWNT−SBP was ca. 75 nM s^{-1}. This value is at least *3 orders of magnitude* higher than that observed with identical enzyme concentrations for either native SBP or SWNT−SBP in the aqueous phase of a biphasic system in the absence of AOT, or for native SBP in the aqueous phase in the presence of AOT. This significant rate enhancement suggests that assembly of SWNT−enzyme conjugates at the aqueous−organic interface can be used to decrease transport limitations typically experienced with biphasic systems and improve the observed catalytic activity.

These enhancements in activity were not obtained with other conventional carbonaceous supports (e.g., graphite flakes), which did not assemble at the interface. While other conventional supports (e.g., hydrophobic nanoporous glass beads (17 nm pore size)) do assemble at the interface in the presence of AOT, the rate of interfacial catalysis is significantly lower (Figure 2), presumably due to intraparticle diffusional limitations.[5] We reasoned that other nonporous nanomaterials might allow enzymes to be transported to the aqueous−organic interface and allow high enzyme loadings due to their high surface area per unit weight, without intraparticle diffusional limitations. Consistent with this hypothesis, hydrophobic octadecyltrimethoxysilane-functionalized silica nanoparticles (SiNPs)

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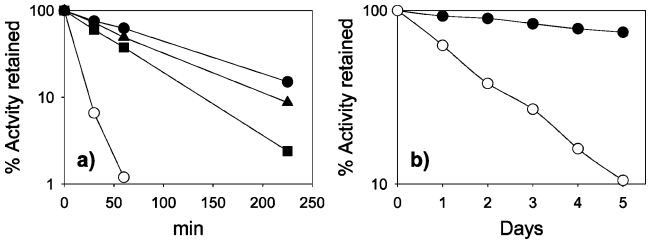

Figure 3. (a) Time-dependent deactivation of SBP in the biphasic system under stagnant conditions: native enzyme (○) and SBP adsorbed onto SWNTs (●), hydrophobic SiNPs (▲), and graphite flakes (■) at 95 °C. (b) Retention of enzymatic activity in the biphasic system at 200 rpm and room temperature: native SBP (○) and SWNT−SBP (●). Each data point represents an average of triplicate measurements with standard error <10%.

were found to assemble at aqueous−organic interfaces in the presence of AOT, transport enzymes to the interface, and also significantly increase the rate of interfacial biocatalysis (Figure 2). A similar enhancement in reactivity was also observed with SiNPs functionalized with (aminopropyl)triethoxysilane (Supporting Information Figure 1). The nanomaterial-mediated interfacial assembly of enzymes, therefore, appears to provide a general route to facilitate interfacial biotransformations.

As expected, the reaction rate for SWNT−SBP conjugates showed a strong dependence on the concentration of *p*-cresol in the hexane phase, with values of $k_{cat} = 5.4\ s^{-1}$ and $K_m = 14.3$ mM (Figure 1c). The reaction rate was also dependent on the enzyme coverage at the interface (Figure 2). At submonolayer coverages, the reaction rate increased linearly with increasing SWNT−SBP interfacial coverage. The specific reaction rate — the reaction rate normalized to the amount of SBP — was constant in this regime. For higher amounts of SWNT−SBP at the interface, the specific reaction rate decreased with increasing amounts of SWNT−SBP at the interface. These results suggest that multiple layers form at the interface for higher amounts of SWNT−SBP, which may limit the rate of diffusion of the reactants across the interface.[6] A similar dependence on surface coverage was seen for conjugates of SBP with SiNPs (Figure 2).

Although the nanotube-mediated interfacial assembly of SWNT−SBP results in significantly higher activity than that in the bulk aqueous phase, the calculated Damköhler number[7] was $\gg 1$, suggesting that the reaction rate may still be limited by mass transfer in the stagnant system employed. Consistent with this hypothesis, the reaction rate increased with increasing shaking speed (see Supporting Information Figure 2), confirming that mass transfer still controls the reaction rate. An additional advantage of higher agitation rates is the corresponding increase in interfacial area in the resulting emulsion system. For higher amounts of SWNT−SBP — those that give multilayer coverage in the stagnant system — more than a 100-fold rate enhancement was achieved in the emulsion system (see Table 1, Supporting Information). For these higher amounts of SWNT−SBP, the increase in interfacial area obtained in the emulsion setup results in submonolayer coverage of SWNT−SBP at the interface, thereby further improving the catalytic efficiency (see Supporting Information).

In addition to enhancing the rate of interfacial biotransformations, the nanotubes also enhance enzyme stability at the interface. Figure 3a reveals that the SWNT−SBP conjugates retained relatively high catalytic activity at the interface, even up to 95 °C. Specifically, the half-life of SWNT−SBP conjugates at 95 °C was ca. 80 min, 8-fold longer than that of the native enzyme in the biphasic system. A similar enhancement in stability was also obtained for SiNP−SBP conjugates, where the half-life was found to be ca. 64 min. Furthermore, the stability on SWNTs is greater than that on SiNPs, which in turn is greater than that on flat graphite flakes, consistent with the differences in curvature of these supports.[4] Figure 3b shows the storage stabilities of native SBP and SWNT−SBP in the aqueous−organic emulsion system at 200 rpm and room temperature. The residual activity of the native SBP after 5 days was ca. 10%. However, the SWNT−SBP retained over 70% of its initial activity over the same period of time.

In conclusion, we have demonstrated the unique intersection of three properties afforded by nanomaterials such as SWNTs: high surface area, ability to assemble at an aqueous−organic interface, and absence of intraparticle diffusional limitations, all of which facilitate interfacial biotransformations. These results are not unique to SBP; horse liver alcohol dehydrogenase adsorbed onto SWNTs is also functional at an aqueous−organic interface, with an activity of ca. 860 nM s^{-1}, while the native enzyme in the two-phase system did not show any measurable activity. Further extension to conjugates of nanotubes or nanoparticles with a broader range of enzymes may result in a new form of biocatalysis where highly efficient biphasic reactions can be performed. Finally, the ability to assemble SWNTs at interfaces provides a route to organize these nanomaterials into 2D architectures — another active area of research.[8]

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Supporting Information Available: Reactivity on APTES-functionalized SiNPs, absolute and specific interfacial reaction rates in the stagnant and emulsion systems, enzyme adsorption onto the different supports, and interfacial activity determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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