DOI: 10.1002/chem.200702010

High Catalytic Activities of Artificial Peroxidases Based on Supramolecular Hydrogels That Contain Heme Models

Qigang Wang, Zhimou Yang, Manlung Ma, Chi K. Chang,* and Bing Xu*[a]

Abstract: Composed of a supramolecular hydrogel and a heme model compound, a new type of artificial peroxidase shows high catalytic activity in organic media. The activity of this new type of artificial enzyme is significantly higher than that of the heme model compounds alone. Changes in the distal substituents above the coordinated-metal centers of the model compounds directly modulate catalytic activity. This supramolecular-hydrogel-based artificial enzyme is most active in toluene, reaching about 90% of the nascent activity of horseradish peroxidase. Moreover, this study confirms that the incorporation of the heme models into the nanofibers of gelators accounts for most of the enhancement of catalytic activity.

Keywords: catalysis · enzyme mimics · gelators · heme models · hydrogels · nanofibers

Introduction

This paper describes the evaluation of the catalytic activity of artificial enzymes composed of a supramolecular hydrogel[1,2,3] and a group of synthetic heme model compounds.[4,5,6] It is well-known that the spatial arrangement of atoms or groups near the active center determines the activities of hemoproteins.[7] To understand the activities of hemes, Chang et al. have synthesized a series of heme models modified by a range of groups that have varying dipole moments and are positioned near the coordinated heme center. From studies on these models, Chang et al. reported that dipolar forces and hydrogen bonding should play a significant role in regulating the oxygen affinities of heme proteins.[5,6] In particular, it was observed that the off-rates of O_2 were governed by the dipole moments in the model molecules equipped with aprotic groups. For the heme-model compounds modified with protic groups, the off-rates of O_2 were determined by hydrogen bonding interactions with the O_2 located at their coordinated centers. Since the off-rates of O_2 from the heme groups in proteins are related to their ability to catalyze peroxidation by affecting the stabilities of the catalytic intermediates, the activities of various hemes likely depends on the nature of the distal groups above the coordinating centers in the heme model compounds.

Supramolecular hydrogels of amphiphilic oligopeptides[8,9] or other small molecules[10–12] are being explored for various important applications, such as scaffolds for tissue engineering,[8] media for screening inhibitors,[11,13] matrices for biomineralization,[14] and biomaterials for wound healing.[12] Similar to the natural selection of peptide chains in enzymes, self-assembled nanofibers of derivatives of amino acids allow the incorporation of heme model compounds as the prosthetic group to mimic peroxidases.[3] In the artificial enzymes reported in this work, the hydrogels serve at least two functions: as the skeleton of the artificial enzyme to aid the function of the active site in organic solvent and as immobilization carriers to facilitate their various applications.

We have demonstrated that the nanofibers in supramolecular hydrogels protect a hemin monomer by preventing its dimerization and degradation and facilitate its catalytic activity by providing nanoporous diffusion channels, which possess unique flexibility to allow the transport of substrates. As a result, the hemin chloride encapsulated in a supramolecular hydrogel reaches about 60% catalytic activity in toluene relative to that of native horseradish peroxidase (HRP) in water.[2] The excellent performance of this enzyme mimic system made of a supramolecular hydrogel prompted us to evaluate whether supramolecular hydrogels could serve as a general platform for the combination of other synthetic heme model compounds and for the deter-

[a] Dr. Q. Wang, Dr. Z. Yang, M. Ma, Prof. C. K. Chang, Prof. B. Xu
Department of Chemistry
The Hong Kong University of Science & Technology
Clear Water Bay, Hong Kong (China)
Fax: (+852) 2358-1594
E-mail: chang@ust.hk
chbingxu@ust.hk

mination of factors that govern or contribute to the catalytic activity in such systems. More specifically, we expected that, similar to the case for the hemoprotein and the trends in catalytic activities observed in the series of model compounds themselves, the distal substituents above the iron centers of the heme model compounds would have a significant influence on the catalytic activities of the supramolecular-hydrogel-based artificial peroxidases.

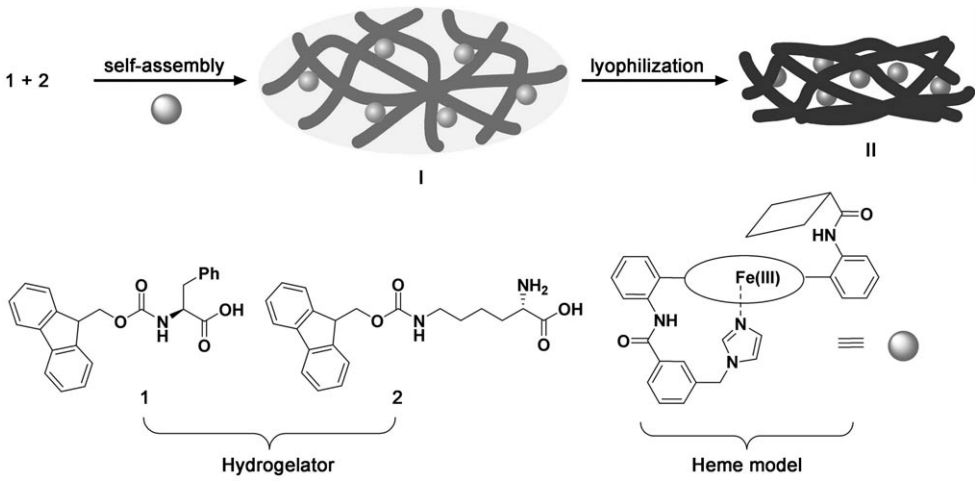
In this work, we found that changes in the distal substituents above the active centers of the heme model compounds also determined the activities of the supramolecular-hydrogel-based artificial enzymes. The highest catalytic activity of the hydrogel-based artificial enzyme (**I**; Scheme 1) in toluene reached about 90% of the activity of HRP in water. By removing water from the hydrogels, we obtained lyophilized artificial enzymes (**II**), which were examined to determine their catalytic activities in organic solvent (toluene). The catalytic activities of the lyophilized enzymes reached up to 75% of that of **I** in organic solvent. These results confirm that supramolecular hydrogels are an excellent platform for making artificial enzymes and verifies the possibility of fine tuning the activity of the artificial peroxidases by changing the distal substituents near the active center of the heme model compounds. This novel approach may provide important guidance for the development of artificial enzymes.

Results and Discussion

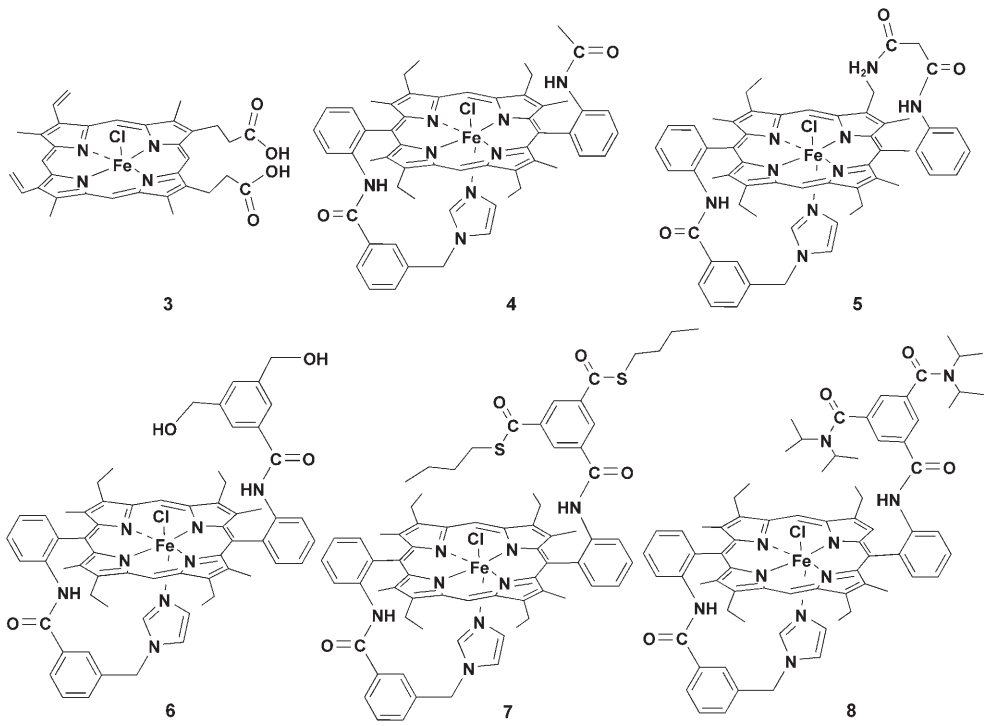
Scheme 1 illustrates the typical procedure for making the artificial peroxidases by using supramolecular hydrogels. The mixture of Fmoc-L-phenylalanine (**1**) (Fmoc: 9-fluorenylmethoxycarbonyl) and Fmoc-L-lysine (**2**) under slightly basic conditions resulted in a suspension, which turned into a clear solution upon heating to 333 K. The addition of one of the heme model compounds **3**–**8**[5] and subsequent cooling afforded a brown, nontransparent hydrogel (**I**). After fast cooling with liquid nitrogen, **I** was freeze-dried to give the lyophilized powder (**II**) containing the nanofibers of **1** and **2**, and the heme model. We characterized both **I** and **II** and investigated their catalytic activities for oxidation.

As shown in the TEM images (Figure 1), the control hydrogel (i.e. that made of only **1** and **2**) and Gel-**6** (i.e. that composed of **1**, **2**, and **6**) have a similar morphology (we chose Gel-**6** as the representative example of Gels-**3** to -**6** as they share the same features). The morphology of the control hydrogel consisted of nanofibers ($\approx$20 nm diameter) of self-assembled **1** and **2**, which were noncovalently entangled to form 50–500 nm pores. Besides the relatively large pores, the diameters of the nanofibers in Gel-**6** are almost the same as those in the control, which suggests that the nanofibers of Gel-**6** mainly consist of **1** and **2**. The gray area/dark particles that surround the nanofibers are probably the heme model compounds and the less-ordered aggregates of **1** and **2**.[3] Moreover, the presence of the heme model compounds in the outer layer of the nanofibers should facilitate the diffusion of the substrates upon their subsequent catalytic application as artificial peroxidases.

Figure 2 shows the UV/Vis spectra of compound **6**, Gel-**6**, and HRP in an aqueous buffer (0.1 M phosphate, pH 7.4). The Soret peak of Gel-**6** at 411 nm is the same as that of pure **6**, which indicates that the configuration of **6** in the artificial peroxidases is unchanged. The Soret peaks of the other compounds (**4**, **5**, **7** and **8**) in the hydrogels are also the same as their corresponding pure forms. However, the Soret peak of compound **3** differs from that of pure **3** in the aqueous buffer because **3** has no axial imidazole-containing ligands for coordination to the iron center. Although it exists in dimer form in the aqueous buffer, in the hydrogel, compound **3** changes into its more active monomeric form as a result of supramolecular interactions. A 1:1 mixture of histidine and Gel **3** shows a redshift in the Soret band (from 400 to 406 nm), which indicates the formation of a hemin–histidine complex stabilized by a Fe^{III}–N bond.[3] In the case of model hemes **4**–**8**, the direct introduction of an axial ligand with imidazole at the *meso* position led to their mon-



Scheme 1. Procedure for making the supramolecular-hydrogel-based artificial enzymes by using hydrogelators and heme model compounds.



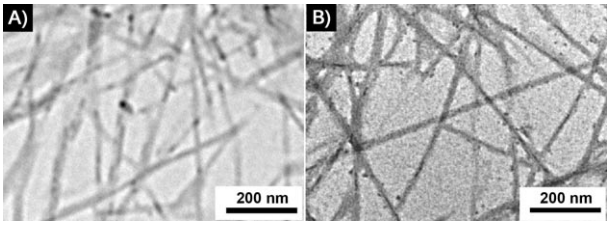


Figure 1. TEM images of the nanofiber matrices in A) the hydrogel made of **1** and **2** without heme model compounds and B) the hydrogel made of **1** and **2** with the addition of compound **6** (Gel-**6**) after cryo-drying.

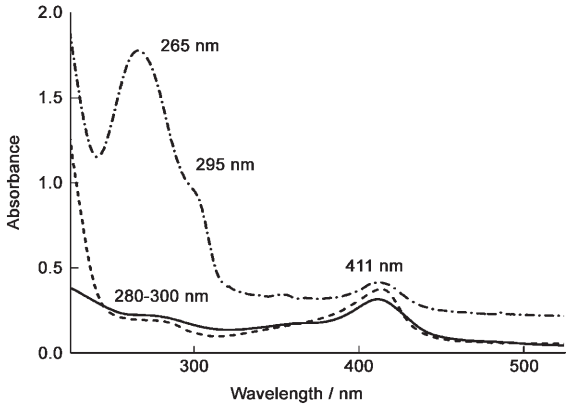


Figure 2. UV/Vis spectra of 4 μM Gel-**6** (–•–•), free **6** (——), and free HRP in pH 7.4 buffer (-----).

omeric forms and produced a redshift of the Soret band relative to that of heme **3**. As a result, the model heme **6** and Gel-**6** have the same Soret band at about 411 nm as that of HRP, which has a similar heme group to that of the designed heme model compounds.[15] Gel-**6** displays another strong peak at 265 nm, which is the characteristic absorbance of the Fmoc groups in **1** and **2**. The absorbance of Gel-**6** at about 295 nm originates from the phenylalanine of the hydrogel. The tryptophan and/or tyrosine residues in HRP lead to the weakly absorbing peak at 280–300 nm.

Using the oxidation of pyrogallol as a model reaction, we monitored the catalytic activities of the artificial peroxidases (Gels-**3** to -**8**) formed by the heme model compounds (**3**–**8**) and the supramolecular hydrogel. As shown in Table 1 and Figure 3, these supramolecular-hydrogel-based, artificial peroxidases all exhibit relatively high activities in toluene that are close to the activities of native peroxidases in water. Relative to Gel-**3**, the artificial enzymes made of Gels-**4** to -**8** have significantly higher activities in toluene. Among them, the activity of Gel-**6** in toluene can reach 90% of that of native HRP in aqueous buffer. These results suggest that hydrogel-encapsulated compounds **4**–**8** have a greater structural similarity to native HRP than Gel-**3**, which is in agreement with the data obtained from their UV/Vis spectra.

The kinetic data in Table 1 indicate that the k_{cat} of Gel-**3** in toluene (370.7 min^{-1}) is 136 times that of free **3** in toluene (2.7 min^{-1}). For model hemes **4**–**8**, the values of k_{cat} for the free forms in toluene are 8.1, 7.5, 9.8, 3.7, and 9.3 min^{-1}, respectively. Compared with the activities of Gels-**4** to -**8** in toluene (1212.2, 1063.6, 1556.9, 545.1, and 1481 min^{-1}), the activity enhancements of the model hemes **4**–**8** after hydrogel immobilization are times 148.6, 140.8, 157.9, 146.3, and 158.3, respectively. The 130–160-fold enhancement of activity for Gels-**3** to -**8** indicates the influence of the novel supramolecular hydrogel skeleton on the artificial peroxidases. Such an effect should be divided into two parts, the effect of

Table 1. The activity data and ratios of various catalytic systems in different solvents.[a]

	k_{cat} in buffer [min^{-1}]	k_{cat} in toluene [min^{-1}]	k_{cat} in toluene after lyophilizing [min^{-1}][b]	Activity ratio[c]
Free **3**	2.4	2.7	2.7	1.00
Gel-**3**	19.9	370.7	255.6	1.45
Gel-**3** + histidine	49.7	1045.3	710.6	1.47
Gel-**4**	59.8	1212.2	776.5	1.56
Gel-**5**	56.6	1063.6	685.4	1.55
Gel-**6**	90.3	1556.9	1146.9	1.35
Gel-**7**	44.4	545.1	406.5	1.34
Gel-**8**	88.6	1481.4	979.5	1.51
HRP	1740.0	1.8	1.8	1.00

[a] The backgrounds of all carriers without heme model compounds have been subtracted. [b] The samples were lyophilized to form a powder before assaying the activities. [c] The ratio of the activity of the artificial enzyme versus the lyophilized artificial enzymes (i.e. dried hydrogels containing heme models).

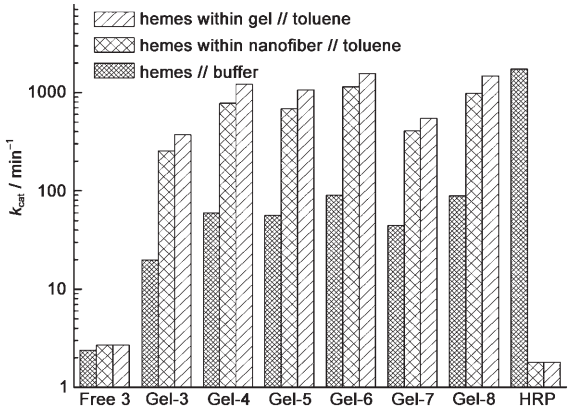


Figure 3. Catalytic activities of the artificial enzymes, heme model compound **3**, and HRP under three different reaction conditions.

the supramolecular nanofibers and that of the phase equilibrium of reactants and products between the hydrophobic toluene solution and the aqueous environment.

By measuring the activities of the lyophilized artificial enzymes in organic media, we can separate the contribution of the amphiphilic nanofibers from the effect of the phase transfer that originates from the organic media and water in the hydrogel. In the case of **3**, the activity of lyophilized Gel-**3** in toluene is about 12 times the activity of Gel-**3** in the aqueous buffer (Table 1). At the same time, the activity of the artificial enzyme (i.e., Gel-**3**) in toluene is about 19 times higher than that of Gel-**3** in the aqueous buffer. Therefore, the effect of phase transfer may contribute to about a 1.5 fold increase in the enhanced activity of the artificial peroxidases in toluene. This enhancement is quite consistent for all the artificial peroxidases. When the heme model compounds are incorporated into the hydrogel, all of the enhancement ratios from phase transfer are about 1.5. Thus, the high activities of the artificial peroxidases in organic solvent mainly originate from the amphililic nanofibers that mimic the molecular environment around the active center in a natural enzyme.

Figure 4 shows the time-course plots of the catalytic reactions over 15 minutes for Gels-**3** to -**8** in water. These data indicate that the activities of artificial peroxidases with different heme model compounds depends on the distal groups above the coordinated iron atoms and reveals that the order of their catalytic abilities in the aqueous buffer is Gel-**6** > Gel-**8** > Gel-**4** > Gel-**5** > Gel-**7** > Gel-**3**. Figure 5 shows the time-course plots of the catalytic reactions over 15 minutes for Gels-**3** to -**8** in toluene, which showed the same order of catalytic activity as in water. Therefore, the order of the activities of the artificial peroxidases is Gel-**6** > Gel-**8** > Gel-**4** > Gel-**5** > Gel-**7** > Gel-**3**. As shown in Figures 5 and 4, the artificial systems have activities in the order of Gels-**6**, -**8**, -**4**, -**5**, -**7** ≫ Gel-**3** in water and Gels-**6**, -**8**, -**4**, and -**5** ≫ Gels-**7** and -**3** in toluene. The major reason for the different reactivities of Gel-**7** in toluene and water is that the hydrophobic alkyl chain on the thioester of **7** is a poor facilitator of hy-

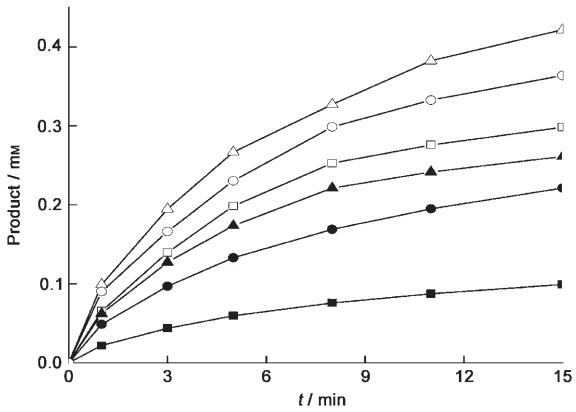


Figure 4. Time-course plots (over 15 minutes) of the reaction of pyrogallol (10.0 mM) and H_2O_2 (40.0 mM) in 0.01 M phosphate buffer at pH 7.4 catalyzed by 5 μM Gel-**3** (■), Gel-**4** (□), Gel-**5** (▲), Gel-**6** (△), Gel-**7** (●), and Gel-**8** (○).

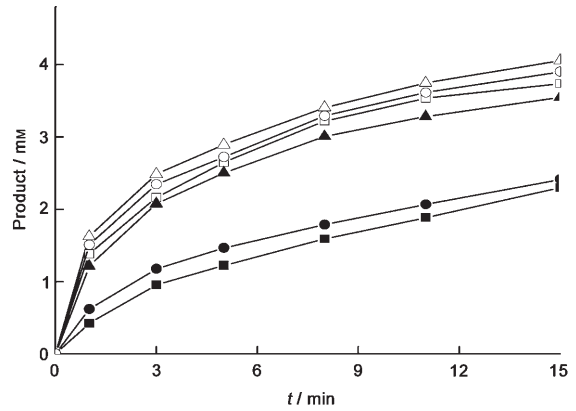
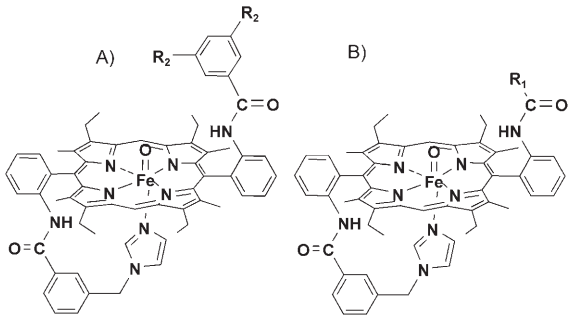


Figure 5. Time-course plots (over 15 minutes) of the reaction of pyrogallol (10.0 mM) and H_2O_2 (40.0 mM) in toluene catalyzed by 5 μM Gel-**3** (■), Gel-**4** (□), Gel-**5** (▲), Gel-**6** (△), Gel-**7** (●), and Gel-**8** (○).

drogen bonding with the iron-bound oxygen atom. When the solvent is water, sufficient water molecules are present to aid the formation of hydrogen bonds on the oxygen atom, thus assisting the catalytic reaction.

It is well-known that during catalysis peroxidases react first with hydrogen peroxide to form intermediate species (Fe^{V}=O–porphyrins).[16] In native HRP, the proximal histidine coordinates to the iron in heme, which can effectively reduce the energy level of the Fe^{V}=O–porphyrin by electron donation from the imidazole group. Therefore, the intermediate species in HRP has lower activation energy and higher activity in the peroxidation reaction. Thus, in an artificial peroxidase, a ligand containing imidazole can have the same positive effect as the histidine group in HRP. This mechanism explains the higher activities of Gels-**4** to -**8** than that of Gel-**3**. The intermediates of the artificial peroxidases formed by the heme model compounds **4**–**8** with an axial ligand containing imidazole might also effectively lower the activation energy.

To explain how the distal groups in heme model compounds can influence the activities of the corresponding artificial peroxidases, we need to examine how the distal groups above the porphyrin ring of the heme model compounds may affect the hydrogen bond formed by the iron–oxo species. The heme model compounds **4**–**8** can be divided into two types. The first type, which includes compounds **6**–**8** (Scheme 2A), has *meta*-substituted benzamides with the



Scheme 2. The orientations of the distal groups in A) compounds **6**–**8** and B) compounds **4** and **5**.

distal groups above the coordination site of the porphyrin ring. The second type, represented by **4** and **5** (Scheme 2B), has the simple *o*-anilide with the distal groups above the coordination site of the porphyrin ring. In the first type, it is apparent that the heme model **6** has distal OH groups and heme model **8** has the tertiary amide near the iron–oxo site of the intermediate. These groups bear lone-pair electrons and may act as hydrogen bond acceptors to lower the activation energy of their intermediates. The higher activation energy of heme model **8** than that of **6** may be ascribed to increased steric hindrance in its molecular structure. For the second type of heme model compound, the electron-deficient distal groups in heme models **4** and **5** are located away from the Fe^{V}=O porphyrin relative to heme models **6** and **8**. As a result, the Fe^{V}=O species from heme models **4** and **5** should have higher activation energies compared with those of heme models **6** and **8**. The higher activation energy of heme model **5** than that of **4** may also be ascribed to increased steric hindrance in its molecular structure. For heme model **7**, the distal alkyl chains from the thioester do not bear lone pair electrons that could act as hydrogen-bond acceptors. Therefore, the intermediate species in **7** has the highest activation energy among the heme models **4**–**8**. Among the artificial peroxidases, Gels-**4** to -**8**, the order of activation energy for their intermediates is Gel-**7** > Gel-**5** > Gel-**4** > Gel-**8** > Gel-**6**, which agrees with the order of activity: Gel-**6** > Gel-**8** > Gel-**4** > Gel-**5** > Gel-**7**.

Conclusions

The combination of a supramolecular hydrogel with various heme model compounds that act as the active center affords artificial peroxidases with high catalytic activities. The activity of the artificial peroxidases can be tailored by changing the distal substituents above the coordinated centers of the heme model compounds, which is independent of the supramolecular hydrogel. Although the effect of phase transfer at the interface of the organic solvent and water in the supramolecular hydrogel provides a certain enhancement, the high activities of the artificial peroxidases in organic solvents are mainly due to the unique features of the amphiphilic nanofibers. Moreover, by using the principles illustrated in this work, it may be possible to tune the activities of the artificial peroxidases by changing the distal substituents or the structure of the hydrogelators. Our future work will expand this general strategy to a variety of artificial enzymes.

Experimental Section

Fmoc-L-phenylalanine (50 μmol), Fmoc-L-lysine (50 μmol), and sodium carbonate (100 μmol) were added to water (1 mL) to afford a suspension, which turned into a clear solution after heating to 333 K. Then, the powdered heme model (10 μmol) was rapidly dissolved in the peptide solution. A hydrogel composite with hemin (Gels-**3** to -**8**) formed after about 10 min. After rapid cooling with liquid nitrogen, **I** was freeze-dried to give the lyophilized powder (**II**). By using the oxidation of pyrogallol (10.0 mM) by H_2O_2 (40.0 mM) as a model reaction and by fixing the total concentration of various hemins at 5 μM in the mixture, the activities of the hemins were obtained by monitoring the absorbance (420 nm) of purpurogallin, the product of hemin-catalyzed oxidation of pyrogallol. The activity constant k_{cat} (V_{max}/[E], in which V_{max} is the velocity of the reaction and [E] is the concentration of the enzyme) was measured by using the initial reaction rate in the first minute at saturated concentration (10.0 mM), which eliminates the effect from the concentration of substrate and enzyme. Therefore, the k_{cat} values in various systems represent the basic kinetic activity.

Acknowledgement

This work was supported by the Hong Kong Research Grant Council (HKU2/05C, HKU2/06C, 604905, 600504, 603907).

[1] S. Sawada, Y. Nomura, Y. Aoyama, K. Akiyoshi, *J. Bioact. Compat. Polym.* **2006**, *21*, 487; L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201; M. George, R. G. Weiss, *Acc. Chem. Res.* **2006**, *39*, 489; Z. M. Yang, G. L. Liang, B. Xu, *Acc. Chem. Res.* **2008**, *41*, 315; P. Terech, R. G. Weiss, *Chem. Rev.* **1997**, *97*, 3133; R. G. Weiss, P. Terech, *Molecular gels: materials with self-assembled fibrillar networks*, Springer, Dordrecht, **2006**.

[2] Q. G. Wang, Z. M. Yang, L. Wang, M. L. Ma, B. Xu, *Chem. Commun.* **2007**, 1032.

[3] Q. G. Wang, Z. M. Yang, X. Q. Zhang, X. D. Xiao, C. K. Chang, B. Xu, *Angew. Chem.* **2007**, *119*, 4363; *Angew. Chem. Int. Ed.* **2007**, *46*, 4285.

[4] C. K. Chang, T. G. Tralor, *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 2647.

[5] C. K. Chang, B. Ward, R. Young, M. P. Kondylis, *J. Macromol. Sci. Chem. A* **1988**, *25*, 1307.

[6] R. Young, C. K. Chang, *J. Am. Chem. Soc.* **1985**, *107*, 898.

[7] P. F. Chen, A. L. Tsai, K. K. Wu, *J. Biol. Chem.* **1994**, *269*, 25062; S. de Lauzon, D. Mansuy, J. P. Mahy, *Eur. J. Biochem.* **2002**, *269*, 470; R. Ricoux, J. L. Boucher, D. Mansuy, J. P. Mahy, *Biochem. Biophys. Res. Commun.* **2000**, *278*, 217; Y. Zhao, J. P. M. Schelvis, G. T. Babcock, M. A. Marletta, *Biochemistry* **1998**, *37*, 4502.

[8] H. A. Behanna, J. J. J. M. Donners, A. C. Gordon, S. I. Stupp, *J. Am. Chem. Soc.* **2005**, *127*, 1193; G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. Harrington, J. A. Kessler, S. I. Stupp, *Science* **2004**, *303*, 1352; S. Vauthey, S. Santoso, H. Y. Gong, N. Watson, S. G. Zhang, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 5355; S. G. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171.

[9] A. Aggeli, M. Bell, N. Boden, J. N. Keen, P. F. Knowles, T. C. B. McLeish, M. Pitkeathly, S. E. Radford, *Nature* **1997**, *386*, 259; E. Gazit, *Chem. Soc. Rev.* **2007**, *36*, 1263; N. Hendler, N. Sidelman, M. Reches, E. Gazit, Y. Rosenberg, S. Richter, *Adv. Mater.* **2007**, *19*, 1485; A. R. Hirst, B. Q. Huang, V. Castelletto, I. W. Hamley, D. K. Smith, *Chem. Eur. J.* **2007**, *13*, 2180; M. A. Kostiainen, G. Z. R. Szilvay, D. K. Smith, M. B. Linder, O. Ikkala, *Angew. Chem.* **2006**, *118*, 3618; *Angew. Chem. Int. Ed.* **2006**, *45*, 3538; P. D. Thornton, R. J. Mart, R. V. Ulijn, *Adv. Mater.* **2007**, *19*, 1252; R. V. Ulijn, *J. Mater. Chem.* **2006**, *16*, 2217.

[10] M. Ikeda, M. Takeuchi, S. Shinkai, *Chem. Commun.* **2003**, 1354; H. Kobayashi, M. Amaike, J. H. Jung, A. Friggeri, S. Shinkai, D. N. Reinhoudt, *Chem. Commun.* **2001**, 1038; S. M. Park, Y. S. Lee, B. H. Kim, *Chem. Commun.* **2003**, 2912; N. Sreenivasachary, J. M. Lehn, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5938; M. Suzuki, M. Yumoto, M. Kimura, H. Shirai, K. Hanabusa, *Chem. Commun.* **2002**, 884; S. Tamaru, S. Kiyonaka, I. Hamachi, *Chem. Eur. J.* **2005**, *11*, 7294; R. V. Ulijn, L. De Martin, L. Gardossi, P. J. Halling, *Curr. Org. Chem.* **2003**, *7*, 1333; B. G. Xing, C. W. Yu, K. H. Chow, P. L. Ho, D. G. Fu, B. Xu, *J. Am. Chem. Soc.* **2002**, *124*, 14846; Z. M. Yang, H. W. Gu, D. G. Fu, P. Gao, K. J. K. Lam, B. Xu, *Adv. Mater.* **2004**, *16*, 1440; Z. M. Yang, H. W. Gu, Y. Zhang, L. Wang, B. Xu, *Chem. Commun.* **2004**, 208; Z. M. Yang, G. L. Liang, L. Wang, B. Xu, *J. Am. Chem. Soc.* **2006**, *128*, 3038; Y. Zhang, Z. M. Yang, F. Yuan, H. W. Gu, P. Gao, B. Xu, *J. Am. Chem. Soc.* **2004**, *126*, 15028; W. Deng, H. Yamaguchi, Y. Takashima, A. Harada, *Angew. Chem.* **2007**, *119*, 5236; *Angew. Chem. Int. Ed.* **2007**, *46*, 5144; B. V. Shankar, A. Patnaik, *J. Phys. Chem. B* **2007**, *111*, 9294; J. E. A. Webb, M. J. Crossley, P. Turner, P. Thordarson, *J. Am. Chem. Soc.* **2007**, *129*, 7155; M. de Loos, A. Friggeri, J. van Esch, R. M. Kellogg, B. L. Feringa, *Org. Biomol. Chem.* **2005**, *3*, 1631; A. Friggeri, C. van der pol, K. J. C. van Bommel, A. Heeres, M. C. A. Stuart, B. L. Feringa, J. van Esch, *Chem. Eur. J.* **2005**, *11*, 5353.

[11] S. Kiyonaka, K. Sada, I. Yoshimura, S. Shinkai, N. Kato, I. Hamachi, *Nat. Mater.* **2004**, *3*, 58; I. Yoshimura, Y. Miyahara, N. Kasagi, H. Yamane, A. Ojida, I. Hamachi, *J. Am. Chem. Soc.* **2004**, *126*, 12204.

[12] Z. M. Yang, K. M. Xu, L. Wang, H. W. Gu, H. Wei, M. J. Zhang, B. Xu, *Chem. Commun.* **2005**, 4414.

[13] Z. M. Yang, B. Xu, *Chem. Commun.* **2004**, 2424.

[14] Z. A. C. Schnepp, R. Gonzalez-McQuire, S. Mann, *Adv. Mater.* **2006**, *18*, 1869.

[15] S. Nagano, M. Tanaka, K. Ishimori, Y. Watanabe, I. Morishima, *Biochemistry* **1996**, *35*, 14251; E. S. Ryabova, A. Dikiy, A. E. Hesslein, M. J. Bjerrum, S. Ciurli, E. Nordlander, *J. Biol. Inorg. Chem.* **2004**, *9*, 385; G. Smulevich, M. Paoli, G. DeSanctis, A. R. Mantini, F. Ascoli, M. Coletta, *Biochemistry* **1997**, *36*, 640.

[16] P. R. O. Demontellano, *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 89; Z. S. Farhangrazi, B. R. Copeland, T. Nakayama, T. Amachi, I. Yamazaki, L. S. Powers, *Biochemistry* **1994**, *33*, 5647; M. A. Gilabert, A. N. P. Hiner, P. A. Garcia-Ruiz, J. Tudela, F. Garcia-Molina, M. Acosta, F. Garcia-Canovasa, J. N. Rodriguez-Lopez, *BBA-Proteins and Proteomics* **2004**, *1699*, 235; R. S. Koduri, M. Tien, *Biochemistry* **1994**, *33*, 4225; J. N. Rodriguez-Lopez, D. J. Lowe, J. Hernandez-Ruiz, A. N. P. Hiner, F. Garcia-Canovas, R. N. F. Thorneley, *J. Am. Chem. Soc.* **2001**, *123*, 11838; A. T. Smith, S. A. Sanders, R. N. F. Thorneley, J. F. Burke, R. R. C. Bray, *Eur. J. Biochem.* **1992**, *207*, 507.

Received: December 20, 2007
Published online: April 9, 2008

