Enzyme Models

A Biomimetic Model for the Active Site of Iron-Only Hydrogenases Covalently Bonded to a Porphyrin Photosensitizer**

Li-Cheng Song, Ming-Yi Tang, Fu-Hai Su, and Qing-Mei Hu*

Hydrogenases are highly efficient enzymes that catalyze the production and consumption of hydrogen reversibly in a wide variety of microorganisms.[1–3] Hydrogenases are generally classified into two major groups depending on their metal content, namely NiFe hydrogenases and Fe-only hydrogenases.[4] Although both groups of hydrogenases catalyze the consumption and production of hydrogen, the Fe-only hydrogenases have much greater reaction rates for H_2 production than NiFe hydrogenases.[5] Therefore, Fe-only hydrogenases (hereafter referred to as FeHases) have received much more attention than NiFe hydrogenases in biomimetic studies

[*] Prof. L.-C. Song, Dr. M.-Y. Tang, Dr. F.-H. Su, Prof. Q.-M. Hu
    Department of Chemistry and
    The State Key Laboratory of Elemento-Organic Chemistry
    Nankai University
    Tianjin 300071 (China)
    Fax: (+86) 22-2350-4853
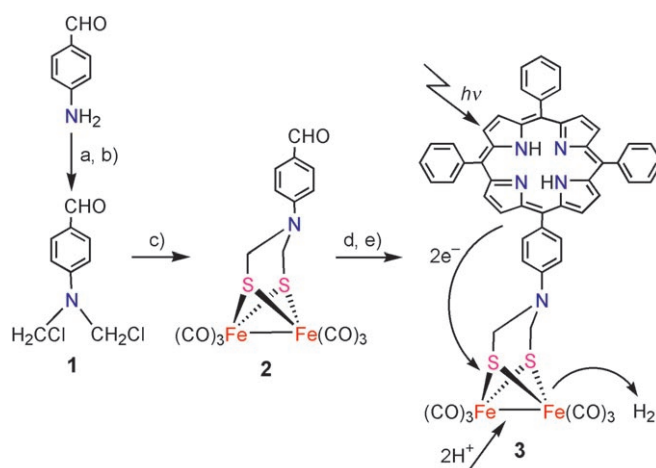   E-mail: lcsong@nankai.edu.cn

aimed at synthesizing model compounds that can act as H_2 production catalysts that are as effective as those found in the natural systems.[6, 7]

Recent X-ray crystallographic[8] and IR spectroscopic[9] studies have indicated that the active site of FeHases, the H-cluster, consists of two iron atoms that are within bonding distance of each other and bridged by a dithiolate ligand. These studies also indicated that there are three other ligands, namely CO, CN^-, and a cysteine-*S*-linked cubic 4Fe4S cluster, coordinated to the two dithiolate-bridged iron atoms; the cubic 4Fe4S cluster is actually the part of the electron transfer chain that links the active site of FeHases. Numerous model compounds have been successfully synthesized and structurally characterized based on the above-mentioned structural studies regarding the active site.[10]

We recently initiated a study on the possibility of linking a porphyrin-type photosensitizer to this simple biomimetic model in order to prepare a new type of light-driven model compounds. Fortunately, we were able to synthesize one such model compound, namely **3** (see Scheme 1), in which a photosensitizing tetraphenylporphyrin group (TPP) is covalently linked to the N atom of the diiron azadithiolate (ADT) moiety [{(μ-SCH$_2$)$_2$N}Fe$_2$(CO)$_6$]. It follows that our light-driven model compound **3** is different from the previously reported cationic model in which a Ru^{II} complex [Ru-(terpy)$_2$]$^{2+}$ is the photosensitizer (terpy = terpyridyl).[11] We chose TPP as the photosensitizer because: 1) TPP can absorb as much as 46% of the energy of sunlight;[12] 2) the lifetime of the excited state of TPP is much longer than those of common Ru^{II}-based photosensitizers;[12] 3) [Ru(terpy)$_2$]$^{2+}$ is cationic, while TPP is neutral; and 4) as a result of the above three points, the expected photoinduced electron transfer in **3** should be easier than in the previously reported cationic model. The ADT-bridged diiron moiety was selected to constitute this model, because it has recently been suggested that the ADT bridge plays an important role in H_2 production in the natural system.[13] In fact, diiron–ADT complexes have proved to be some of the best simple models for the active site of FeHases.[10c,f]

The synthetic route to **3** and its expected light-driven process are briefly illustrated in Scheme 1. First, *N,N*-di(chloromethyl)-4-formylaniline (**1**) is prepared by treatment of *p*-aminobenzaldehyde with paraformaldehyde and then with $SOCl_2$.[14] Second, compound **1** was treated with di-μ-sulfanylbis(tricarbonyliron)[15] to afford the simple *p*-benzaldehyde-substituted model **2**. Finally, the target model **3** was synthesized by treatment of **2** with pyrrole and benzaldehyde in a 1:4:3 molar ratio followed by the oxidant *p*-chloranil.[16] In the expected light-driven process, model **3** should first absorb a photon at the photosensitizer TPP. This photoexcited TPP is then oxidatively quenched by the diiron unit to give a reduced iron species. After regeneration of the TPP by transfer of an electron from an external donor, this process is repeated to produce a doubly reduced diiron species that should be able to drive the reduction of protons to hydrogen (Scheme 1).

The new compounds **1–3** were characterized by elemental analysis and various spectroscopic techniques. The IR spectrum of **3** shows three absorption bands at 1558, 1472, and 1350 cm^{-1}, assigned to the skeletal vibrations of the pyrrole

Scheme 1. a) (CH$_2$O)$_n$, CH$_2$Cl$_2$, room temperature, 5 h; b) SOCl$_2$, room temperature, 1 h; c) [(μ-HS)$_2$Fe$_2$(CO)$_6$], THF, room temperature, 12 h; d) PhCHO, pyrrole, CF$_3$CO$_2$H, CH$_2$Cl$_2$, room temperature, 15 h; e) *p*-chloranil, reflux, 1 h.

rings in the porphyrin unit,[17] and strong absorption bands at 2073, 2033, and 1996 cm^{-1} due to the terminal CO groups in the diiron–ADT moiety. The ^{1}H NMR spectrum of **3** displays a singlet at $\delta = 4.55$ ppm for the CH$_2$ groups in the diiron–ADT moiety and another singlet at $\delta = -2.76$ ppm for the NH groups of the porphyrin unit.[18] The electronic absorption spectra of **3** and TPP were also recorded. As shown in Figure 1, there is one Soret band at 419 nm in the near-UV
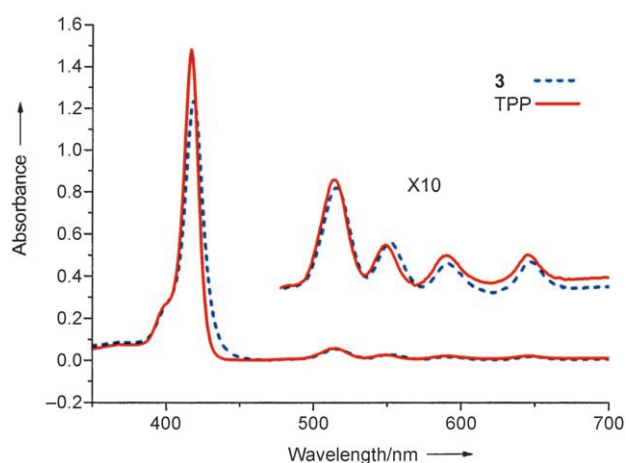

Figure 1. UV/Vis spectra of **3** and TPP in CH$_2$Cl$_2$ (3×10^{-6} M). The Soret band absorptions have been normalized and the spectra amplified 10-fold in the Q band region.

region and four Q bands at 516, 552, 592, and 647 nm in the visible region.[19] These Soret and Q bands are red-shifted by only 1–3 nm relative to the corresponding bands of TPP under the same conditions.

The fluorescence emission spectrum of **3**, along with those of TPP and an equimolar mixture of TPP and [{(μ-SCH$_2$)$_2$NH}Fe$_2$(CO)$_6$], were also recorded. As shown in Figure 2, the two fluorescence emission bands at 655 and 719 nm displayed by **3** are red-shifted by about 3 nm relative
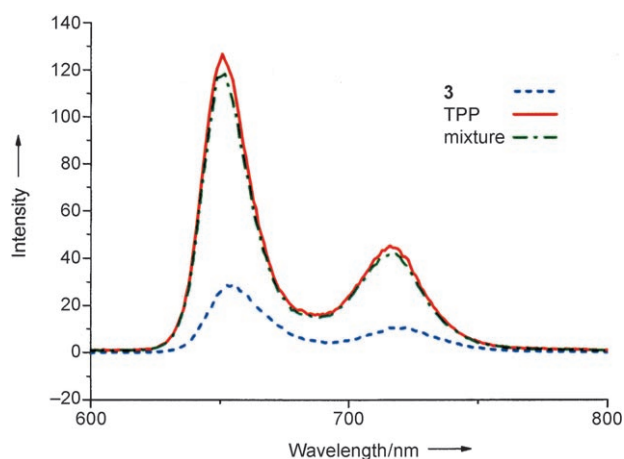
Figure 2. Fluorescence emission spectra ($\lambda_{ex} = 425$ nm) of **3**, TPP, and an equimolar mixture of TPP and $[\{(\mu\text{-SCH}_2)_2NH\}Fe_2(CO)_6]$ in CH_2Cl_2 (3×10^{-5} M).

to the corresponding bands of TPP under the same conditions, although their intensities are strongly quenched relative to those of the TPP bands, with a quenching efficiency, Q, of 86.4%. Since the intensities of the two bands of the equimolar mixture of TPP and $[\{(\mu\text{-SCH}_2)_2NH\}Fe_2(CO)_6]$ are almost the same as those of the corresponding TPP bands, we believe that the remarkably decreased intensities of the two fluorescence bands of **3** relative to those of TPP are most likely due to strong intramolecular electron transfer from the photoexcited state of the porphyrin macrocycle of TPP to the covalently bonded diiron–ADT moiety and not by an intermolecular collision process between molecules of **3**.[20] It is interesting to note that such an intramolecular electron transfer is one of the important steps required for the proton reduction performed by biomimetic models.

The molecular structures of model **3** and its precursor **2** were confirmed by X-ray crystallography (Figures 3 and 4, respectively).[21] Both **2** and **3** contain a diiron–ADT moiety in which the six-membered ring Fe1-S1-C7-N1-C8-S2 has a boat conformation and the other six-membered ring Fe2-S1-C7-N1-C8-S2 adopts a chair conformation. The bond between the disubstituted benzene ring and the N1 atom is axially oriented in both **2** and **3**, and all the iron nuclei in **2** and **3** have the expected square-pyramidal coordination sphere. The Fe–Fe bond lengths of 2.4956(9) Å for **2** and 2.5000(9) Å for **3** are in good agreement with the corresponding lengths in similar complexes.[10] The sum of the C-N-C angles around N1 is 356.8° for **2** and 355.3° for **3**, which implies somewhat interrupted π conjugation between the disubstituted benzene ring and the p orbital of the nitrogen atom.

While **2** is a simple model compound where the nitrogen atom is linked to an aromatic aldehyde system, the target compound **3** is a novel light-driven-type model with a porphyrin photosensitizer. The C–N bond lengths in the pyrrole rings of the porphyrin unit of **3** are nearly the same (1.364(5)–1.378(5) Å) and lie between those of normal single and double C–N bonds.[22] The four benzene rings attached to the porphyrin unit of **3** are planar and twisted relative to the porphyrin plane in order to reduce the steric interactions
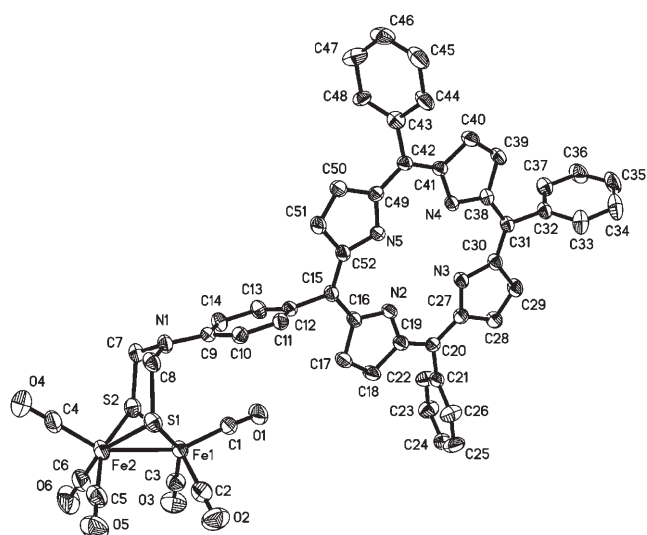

Figure 3. Structure of **3**. Selected bond lengths [Å]: Fe1–Fe2 2.5000(9), Fe1–S1 2.2630(13), Fe1–S2 2.2607(15), N1–C7 1.428(6), N1–C8 1.413(6), C12–C15 1.503(6), C16–N2 1.367(5), C27–N3 1.375(5), C38–N4 1.373(5), C49–N5 1.370(6).
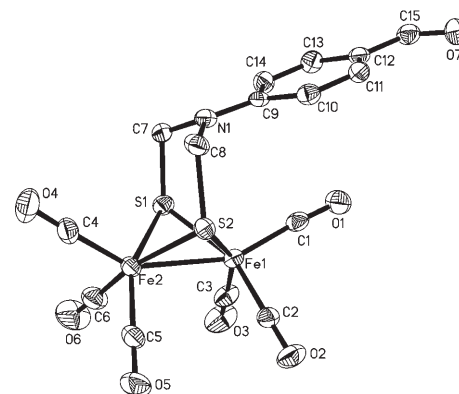

Figure 4. Structure of **2**. Selected bond lengths [Å]: Fe1–Fe2 2.4956(9), Fe1–S1 2.2542(12), Fe1–S2 2.2417(11), Fe2–S1 2.2486(11), N1–C7 1.424(4), N1–C8 1.431(4), N1–C9 1.387(4), O7–C15 1.193(4).

between the phenyl hydrogen atoms proximal to the porphyrin unit and the pyrrole rings. The twist angles between the porphyrin plane and the benzene rings involving C12, C21, C32, and C43 are 75.9°, 99.1°, 77.4°, and 63.1°, respectively.

In conclusion, we have synthesized and crystallographically characterized a novel light-driven-type model compound (**3**) that contains a TPP photosensitizer covalently bonded to the N atom of a diiron–ADT moiety. In view of the strong coordination ability of porphyrin macrocycles with a wide variety of metal ions[12] and the facile substitution of the Fe-bound CO by other ligands,[23] we expect that we will be able to further modify **3** in order to facilitate the electron transfer from the photoexcited TPP to the diiron–ADT moiety and thus finally accomplish the light-driven reduction of protons to hydrogen. Further investigations in this area are in progress.

Experimental Section

1: A mixture of p-$NH_2C_6H_4CHO$ (1.265 g, 10.4 mmol), paraformaldehyde (1.14 g), and CH_2Cl_2 (20 mL) was stirred at room temperature for 5 h. After addition of $SOCl_2$ (3.0 mL, 41.2 mmol), the new mixture was stirred for an additional hour. The solvent and unreacted $SOCl_2$ were then removed under vacuum. The residue was extracted with Et_2O and recrystallized to give 1.383 g (61%) of **1** as a yellow solid. $C_9H_9Cl_2NO$: calcd. C 49.57, H 4.16, N 6.42; found C 49.67, H 4.37, N 6.37; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 5.54$ (s, 4H; $2CH_2$), 7.31, 7.91 (2 d, $J = 8.7$ Hz, 4H; C_6H_4), 9.94 ppm (s, 1H; CHO); IR (KBr disk): $\tilde{\nu} = 1694$ cm^{-1} (CHO).

2: A solution of $Li(Et_3BH)$ in THF (1 M, 17.0 mL, 17.0 mmol) was added to a solution of $[(\mu\text{-}S_2)Fe_2(CO)_6]$ (2.924 g, 8.5 mmol) in THF (40 mL) at $-78\,°C$. At the midpoint of the addition, the reaction mixture turned from red to dark green; for the rest of addition it remained green. CF_3CO_2H (1.3 mL, 17.0 mmol) was then added to give a red solution of $[(\mu\text{-}HS)_2Fe_2(CO)_6]$.[15] Compound **1** (1.845 g, 8.5 mmol) was added to this solution and the mixture was stirred at $-78\,°C$ for 0.5 h and at room temperature for 12 h. The volatiles were removed under vacuum and the residue was separated by TLC using CH_2Cl_2/petroleum ether (2:1, v/v) as eluent. Complex **2** was obtained as a red solid from the major, red band (1.320 g, 32%). $C_{15}H_9Fe_2NO_7S_2$: calcd. C 36.69, H 1.85, N 2.85; found C 36.75, H 1.80, N 3.02; ^1H NMR (300 MHz, $CDCl_3$): $\delta = 4.36$ (s, 4H; $2CH_2$), 6.83, 7.85 (2 d, $J = 8.4$ Hz, 4H; C_6H_4), 9.86 ppm (s, 1H; CHO); IR (KBr disk): $\tilde{\nu} = 2084, 2034, 2007, 1987, 1966$ (C≡O), 1666 cm^{-1} (CHO).

3: A mixture of benzaldehyde (0.182 mL, 1.8 mmol), **2** (0.295 g, 0.6 mmol), pyrrole (0.166 mL, 2.4 mmol), CF_3CO_2H (0.184 mL, 2.4 mmol), and CH_2Cl_2 (240 mL) was stirred at room temperature in the dark for 15 h to give a purple-red solution. 2,3,5,6-Tetrachlorobenzoquinone (p-chloranil; 0.443 g, 1.8 mmol) was added to this solution and the new mixture was refluxed for 1 h to give a brown-green solution. The solvent was removed under reduced pressure and the residue was subjected to flash column chromatography (Al_2O_3, CH_2Cl_2). The eluate was reduced to a suitable volume for TLC separation with CH_2Cl_2/petroleum ether (1:1, v/v) as eluent. Tetraphenylporphyrin (0.052 g, 19%) was obtained from the first band as a purple solid that was identified by comparison of its IR and ^1H NMR spectra with those of an authentic sample.[17,18] Complex **3** (0.138 g, 23%) was obtained as a purple-red solid from the second band. $C_{52}H_{33}Fe_2N_5O_6S_2$: calcd. C 62.48, H 3.37, N 7.01; found C 62.39, H 3.32, N 7.04; ^1H NMR (300 MHz, $CDCl_3$): $\delta = -2.76$ (s, 2H; 2NH), 4.55 (s, 4H; $2CH_2$), 7.04, 7.07 (2 s, 2H; 2 m-H of C_6H_4), 7.74, 7.76 (2 s, 9H; 6 m-H of C_6H_5 and 3 p-H of C_6H_5), 8.11, 8.14 (2 s, 2H; 2 o-H of C_6H_4), 8.20, 8.23 (2 s, 6H; 6 o-H of C_6H_5), 8.85, 8.86 ppm (2 s, 8H; pyrrole rings); IR (KBr disk): $\tilde{\nu} = 3317$ (NH), 2073, 2033, 1996 (C≡O), 1558 (pyrrole C=C), 1472 (pyrrole C=N), 1350 cm^{-1} (pyrrole C−N); ESI-MS: m/z 999.85 [M^+]; UV/Vis (CH_2Cl_2): λ_{max} (log ε) = 419 (5.61), 516 (4.24), 552 (3.93), 592 (3.72), 647 nm (3.74).

[1] R. Cammack, *Nature* **1999**, *397*, 214–215.

[2] M. W. W. Adams, E. I. Stiefel, *Science* **1998**, *282*, 1842–1843.

[3] M. Y. Darensbourg, E. J. Lyon, J. J. Smee, *Coord. Chem. Rev.* **2000**, *206–207*, 533–561.

[4] M. Frey, *ChemBioChem* **2002**, *3*, 153–160.

[5] M. W. W. Adams, *Biochim. Biophys. Acta* **1990**, *1020*, 115–145.

[6] J. Alper, *Science* **2003**, *299*, 1686–1687.

[7] D. J. Evans, C. J. Pickett, *Chem. Soc. Rev.* **2003**, *32*, 268–275.

[8] a) J. W. Peters, W. N. Lanzilotta, B. J. Lemon, L. C. Seefeldt, *Science* **1998**, *282*, 1853–1858; b) Y. Nicolet, C. Piras, P. Legrand, C. E. Hatchikian, J. C. Fontecilla-Camps, *Structure* **1999**, *7*, 13–23.

[9] a) A. L. de Lacey, C. Stadler, C. Cavazza, E. C. Hatchikian, V. M. Fernandez, *J. Am. Chem. Soc.* **2000**, *122*, 11 232–11 233; b) Z. Chen, B. J. Lemon, S. Huang, D. J. Swartz, J. W. Peters, K. A. Bagley, *Biochemistry* **2002**, *41*, 2036–2043.

[10] See, for example: a) F. Gloaguen, J. D. Lawrence, M. Schmidt, S. R. Wilson, T. B. Rauchfuss, *J. Am. Chem. Soc.* **2001**, *123*, 12 518–12 527; b) M. Razavet, S. C. Davies, D. L. Hughes, J. E. Barclay, D. J. Evans, S. A. Fairhurst, X. Liu, C. J. Pickett, *Dalton Trans.* **2003**, 586–595; c) J. D. Lawrence, H. Li, T. B. Rauchfuss, M. Bénard, M.-M. Rohmer, *Angew. Chem.* **2001**, *113*, 1818–1821; *Angew. Chem. Int. Ed.* **2001**, *40*, 1768–1771; d) H. Li, T. B. Rauchfuss, *J. Am. Chem. Soc.* **2002**, *124*, 726–727; e) L.-C. Song, Z.-Y. Yang, H.-Z. Bian, Q.-M. Hu, *Organometallics* **2004**, *23*, 3082–3084; f) S. Ott, M. Kritikos, B. Åkermark, L. Sun, R. Lomoth, *Angew. Chem.* **2004**, *116*, 1024–1027; *Angew. Chem. Int. Ed.* **2004**, *43*, 1006–1009.

[11] S. Ott, M. Kritikos, B. Åkermark, L. Sun, *Angew. Chem.* **2003**, *115*, 3407–3410; *Angew. Chem. Int. Ed.* **2003**, *42*, 3285–3288.

[12] a) J. R. Darwent, P. Douglas, A. Harriman, G. Porter, M.-C. Richoux, *Coord. Chem. Rev.* **1982**, *44*, 83–126; b) I. Okura, *Coord. Chem. Rev.* **1985**, *68*, 53–99.

[13] a) Y. Nicolet, A. L. de Lacey, X. Vernède, V. M. Fernandez, E. C. Hatchikian, J. C. Fontecilla-Camps, *J. Am. Chem. Soc.* **2001**, *123*, 1596–1601; b) H.-J. Fan, M. B. Hall, *J. Am. Chem. Soc.* **2001**, *123*, 3828–3829.

[14] J. D. Lawrence, H. Li, T. B. Rauchfuss, *Chem. Commun.* **2001**, 1482–1483.

[15] D. Seyferth, R. S. Henderson, L.-C. Song, *J. Organomet. Chem.* **1980**, *192*, C1–C5.

[16] J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney, A. M. Marguerettaz, *J. Org. Chem.* **1987**, *52*, 827–836.

[17] D. W. Thomas, A. E. Martell, *J. Am. Chem. Soc.* **1959**, *81*, 5111–5119.

[18] B. C. Bookser, T. C. Bruice, *J. Am. Chem. Soc.* **1991**, *113*, 4208–4218.

[19] J. L. Shaw, S. A. Garrison, E. A. Alemán, C. J. Ziegler, D. A. Modarelli, *J. Org. Chem.* **2004**, *69*, 7423–7427.

[20] a) M. Dudiè, P. Lhoták, V. Král, K. Lang, I. Stibor, *Tetrahedron Lett.* **1999**, *40*, 5949–5952; b) D. Baskaran, J. W. Mays, X. P. Zhang, M. S. Bratcher, *J. Am. Chem. Soc.* **2005**, *127*, 6916–6917.

[21] CCDC-284275 (**2**) and -284276 (**3**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif..

[22] a) S. J. Silvers, A. Tulinsky, *J. Am. Chem. Soc.* **1967**, *89*, 3331–3337; b) J. L. Sessler, M. R. Johnson, S. E. Creager, J. C. Fettinger, J. A. Ibers, *J. Am. Chem. Soc.* **1990**, *112*, 9310–9329.

[23] L.-C. Song, *Acc. Chem. Res.* **2005**, *38*, 21–28.