Design and Synthesis of New Biomimetic Materials by Sol−Gel: A $Cu^{II}(histidine)_2$ Complex Covalently Bonded on a Silica Matrix

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The synthesis of a new histidine-silane derivative, Boc−His(Boc)−CONH−$(CH_2)_3Si(OEt)_3$, is reported. Hydrolysis and co-condensation of this monomer with tetraethoxysilane, via the sol−gel procedure, results in a hybrid inorganic−organic material that bears histidine molecules covalently bonded on a silica matrix. 1D-ESEEM and 2D-HYSCORE studies of its Cu(II) complex show that the copper atom is coordinated by two inequivalent histidine imidazoles. The new Cu(II) material exhibits catalytic activity for DTBQ formation in the presence of dioxygen, with considerable turnover rates and yields. In addition it is highly recyclable and shows high specific surface area.

Introduction

In recent years, much attention has been focused on the activation of dioxygen by monooxygenases.[1a] Copper−dioxygen interactions occur in proteins such as tyrosinase and dopamine β-hydroxylase.[1b,c] In these systems, the copper ions are coordinated by one or more imidazole residues of histidine.[1] The ability of various copper complexes to catalyze biomimetic oxidation processes has been investigated, in addition to searching for models of copper protein active sites and to deduce the mechanism of O_2 activation.[1]

As far as biomimetic oxidations are concerned, an approach toward more easily recovered catalysts and more regioselective systems is to support the biomimetic catalysts on inorganic or organic polymers. Metal catalysts can be heterogenized first, with no direct bonding to the matrix, either by their simple absorption[2a] and intercalation[2a,b] into clays or by encapsulation into zeolite cages.[2c−f] Second, an alternative procedure involves their anchoring via a coordination[3a−c] or a covalent bond on either inorganic[3d−f] or organic supports.[3g−k] More specifically, the immobilization of porphyrin analogues had been proven to be a fruitful strategy toward the biomimetic oxidations of heme-enzymes, like cytochrome P450 and peroxidases.[4] An attractive extension of this idea is the immobilization of the active-center analogues of nonheme enzymes. In this context, we report here a device and easy procedure for covalently anchoring copper−amino acid complexes on a silica support, prepared in situ by the sol−gel procedure.[5]

Electron spin−echo envelope modulation (ESEEM) spectroscopy, which is a pulsed EPR technique, is eminently suited for measuring weak hyperfine couplings and has been proven to be a powerful technique for the study of copper(II) complexes in proteins as well as in model systems.[6] In addition the hyperfine sublevel correlation (HYSCORE) spectroscopy, which is a 2D four-pulse ESEEM technique, offers a tool for the proper analysis of complicated overlapping ESEEM spectra.[7] Here we have used these techniques to study the coordination environment of the copper(II) complexes in the new material.

Our approach intended to build hybrid organic−inorganic materials in which *amino acids* (histidine in this case) form part

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of the network, in the hope to mimic various copper enzymes. Thus, the new prepared material, $[His-CONH(CH_2)_3SiO_{3/2}]_n \cdot xSiO_2$, contains an organic part (amino acids) and an inorganic support (silica). Its copper(II) complex exhibits high specific surface area and, although it contains mononuclear copper(II) centers according to the EPR spectra, shows catecholase activity.

Experimental Section

Materials and Methods. Reagents were purchased from Sigma-Aldrich Co. THF and ether were dried by standard methods and were distilled under nitrogen immediately prior to use. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Pseudo-peptide purity was controlled by thin-layer chromatography (TLC) in the systems $CHCl_3$/MeOH (95:5 v/v) and AcOH/H_2O (1:9 v/v). Column chromatography employed silica gel G15 from E. Merck.

Preparations. Boc−His(Boc)CONH(CH$_2$)$_3$Si(OEt)$_3$ (2a). *N*-Methylmorpholine (N-MM) (0.15 g, 1.5 mmol) and isobutyl chloroformate (IBCF) (0.19 g, 1.4 mmol) were added to a cooled (−15 °C), stirred solution of Boc−His(Boc)OH (0.5 g, 1.4 mmol) in THF (15 mL). Then the solution was stirred at −15 °C for 3 min (to generate the reactive intermediate Boc−His(Boc)COO−$COOCH_2CH(CH_3)_2$ (**1a**). Subsequently (3-aminopropyl)triethoxysilane (APTES) (0.10 g, 0.45 mmol) was added, and the solution was stirred at −10 °C for 1 h and at 0 °C for 2 h. The reaction was carried out in a ratio 3:1 (**1a**: (3-aminoprolyl)-triethoxysilane) according to the practice of solid-phase peptide synthesis. The cold mixture was quickly filtered through Celite, and the solvent was evaporated under inert atmosphere. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98(1H, s), 7.15(1H, s), 4.08(1H, t), 3.10(2H, m), 3.65-(2H, t), 1.55(2H, m), 0.55(2H, t), 1.35(18H, s), 3.72(6H, m), 1.14(9H, t).

[Boc−His(Boc)CONH(CH$_2$)$_3$SiO$_{3/2}$]$_n$·xSiO$_2$ (3a). A solution of tetraethoxysilane (TEOS) (2.1 g, 10 mmol) and monomer **2a** (without isolation from the reaction mixture) in 15 mL of H_2O in the presence of NaF as catalyst was stirred for 48 h at room temperature. The resulting material **3a** was filtered and washed exhaustively with water and various organic solvents in order to eliminate the excess of uncoupled amino acid. More particularly, it was washed with H_2O, H_2O/MeOH = 1:1 v/v, MeOH, CH_3COCH_3, and CH_2Cl_2, extracted in a Soxhlet apparatus with CH_2Cl_2, and dried at 80 °C for 2 h. Detection of free amino groups by the Kaiser test[8] was negative.

[HisCONH(CH$_2$)$_3$SiO$_{3/2}$]$_n$·xSiO$_2$ (4a). Treatment of **3a** with a solution of 40% CF_3COOH/CH_2Cl_2 in the presence of 1% anisole (3 mL for 5 min), subsequent washing with H_2O, H_2O/MeOH = 1:1 v/v, and CH_2Cl_2, and repeated treatment (3 mL for 10 min) and washing by the same manner gave the material **4a**. The presence of free amino groups and N_{im} nitrogens in the **4a** was detected by the Kaiser and Pauly tests, respectively.[8] Amino acid loading was obtained by measuring the amount of C and N in the resulting material, and it was found to be 7.6% (m/m). Anal. Found for **4a**: C, 6.96; N, 2.17.

Cu(His∼)$_2$ (5a). The pH of a solution of **4a** (0.2 g) in H_2O was adjusted to 7, and then $CuCl_2$ (0.02 g, 0.2 mmol) was added. The suspension was stirred for 48 h at room temperature, and the resulting blue material **5a** was filtered, washed with H_2O, CH_3COCH_3, and Et_2O, and dried at 80 °C for 2 h. The amount of Cu^{II} was determined by back-titration of the remaining amount of Cu^{II} into the solution. Amino acid loading was obtained by measuring the amount of C and N in the resulting material, and it was found to be 6.6% (m/m). Anal. Found for **5a**: C, 6.09; N, 1.89; Cu, 1.0.

HisCONH(CH$_2$)$_2$CH$_3$ (4b) and Cu[HisCONH(CH$_2$)$_2$CH$_3$]$_2$Cl$_2$ (5b). *n*-Propylamine (0.06 g, 1.1 mmol) was added to a cooled (−15 °C) solution of reactive intermediate Boc−His(Boc)COO−$COOCH_2$-$CH(CH_3)_2$ (**1a**) (generated as previously described). After stirring at −10 °C for 1 h and at 0 °C for 2 h, the solvent was evaporated, the residue was dissolved in ethyl acetate, and the solution was washed successively with 10% citric acid, water, 10% $NaHCO_3$, and brine. After evaporation of the solvent, 3 mL of HCl/dioxane was added to the residue **2b**, and the solution was stirred for 2 h. Precipitation by anhydrous ether and purification by column chromatography (packed with G15 and eluted with water) gave **4b**: ^{1}H NMR (400 MHz, D_2O, pD = 2.6) δ 8.73(1H, s), 7.45(1H, s), 4.21(1H, t), 3.15(2H, m), 3.38-(2H, t), 1.41(2H, m), 0.77(3H, t); ^{1}H NMR (400 MHz, D_2O, pD = 7.0) δ 7.85(1H, s), 7.06(1H, s), 3.99(1H, t), 3.18(2H, m), 3.09(2H, t), 1.42(2H, m), 0.78(3H, t). The pH adjusting of an aqueous solution of **4b** (0.1 g, 0.48 mmol) to 7 by addition of 0.1 N KOH was followed by evaporation of the water and dissolution of the residue in a mixture of MeOH/CH_3COCH_3 = 1:1 v/v. After the filtering off the salt formed, $CuCl_2$ (0.03 g, 0.24 mmol) was added into the filtrate and the solution was stirred for 24 h at room temperature. Evaporation and one recrystallization from MeOH−ether gave **5b**: λ/nm (H_2O) 714; EPR (MeOH) $g_{II} = 2.29$, $g_{\perp} = 2.05$, $A_{II} = 161$ G.

Instrumentation. NMR spectra at 400 MHz were recorded at room temperature on a Bruker AMX 400 spectrometer with TMS as an internal standard.

Specific surface areas of the materials were determined from N_2 adsorption measurements using a Carlo Erba Sorpty 1750 analyzer.

Diffuse reflectance UV−vis spectra were recorded at room temperature on a Perkin-Elmer Lambda 9 UV−vis spectrometer fitted with a powder attachment.

UV−vis spectra were recorded using a UV/VIS/NIR JASCO spectrophotometer.

GC analysis was performed using a 8000 Fisons chromatograph with a flame ionization detector and a DB WAX column.

EPR and ESEEM Spectra. Continuous-wave (CW) EPR spectra were recorded at liquid helium temperatures with a Bruker ER 200D X-band spectrometer equipped with an Oxford Instruments cryostat. The microwave frequency and the magnetic field were measured with a microwave frequency counter HP 5350B and a Bruker ER035M NMR-gaussmeter, respectively. Pulsed EPR was performed with a Bruker ESP380 spectrometer with a dielectric resonator.[9a] In the three-pulse ($\pi/2-\tau-\pi/2-T-\pi/2$) ESEEM data the amplitude of the stimulated echo as a function of $\tau+T$ was measured at a frequency near 9.6 GHz at a magnetic field corresponding to the maximum intensity of the field-swept spectrum. Data manipulations were performed as described earlier.[9a]

ESEEM Simulations: ^{14}N ($S = 1/2$, $I = 1$). The spin Hamiltonian for an $S = 1/2$, $I = 1$ system expressed in the **g**-tensor principal axes system is

$$H = (\beta/h)B\,\mathbf{g}\,S - g_N(\beta/h)BI + S\cdot\mathbf{A}'\cdot I + I\cdot\mathbf{Q}'\cdot I \qquad (1)$$

The **g**-tensor has principal values (g_x, g_y, g_z), and the Euler angles α, β, γ relate the principal axes system of the **A**′- and **g**-tensors.[9a] The polar angles θ and ϕ determine the orientation of the applied magnetic field in the **g**-tensor principal axes according to standard notation. The hyperfine tensor **A**′ in eq 1 is defined as **A**′= **A g**, where the diagonal values of the **A**-tensor in its principal axes system are (A_{xx}, A_{yy}, A_{zz}). The Euler angles (α, β, γ) and (u, v, w) relate the principal axes systems of tensors **A**′ and **Q**′ to that of **g**, respectively. The energies and eigenfunctions are calculated numerically for each spin manifold, and the orientation selective three-pulse modulations E_α and E_β were calculated with the relations derived by Mims[9b] as described earlier.[9a]

Kinetic Studies. Reaction rates of DTBQ formation were monitored by UV−vis spectroscopy by the appearance of its characteristic band at 390 nm (ϵ 1730 dm^3 mol^{-1} cm^{-1} in MeOH). The metal complex (0.3 mL of a 10^{-3} M methanol solution), 30 μL of triethylamine (10^{-1} N methanol solution), and a 2.0 mL solution (10^{-1} M methanol solution) of 3,5-di-*tert*-butylcatechol were added together in the spectrophotometric cell at 25 °C.

Oxidations Reactions. In a typical experiment, catalyst complex (0.3 mL of a 10^{-3} M methanol solution or 2.4 mg of the material) and 30 μL (or 300 μL) of triethylamine (10^{-1} N methanol solution) were mixed with a 2.0 mL solution (10^{-1} M methanol solution) of 3,5-di-

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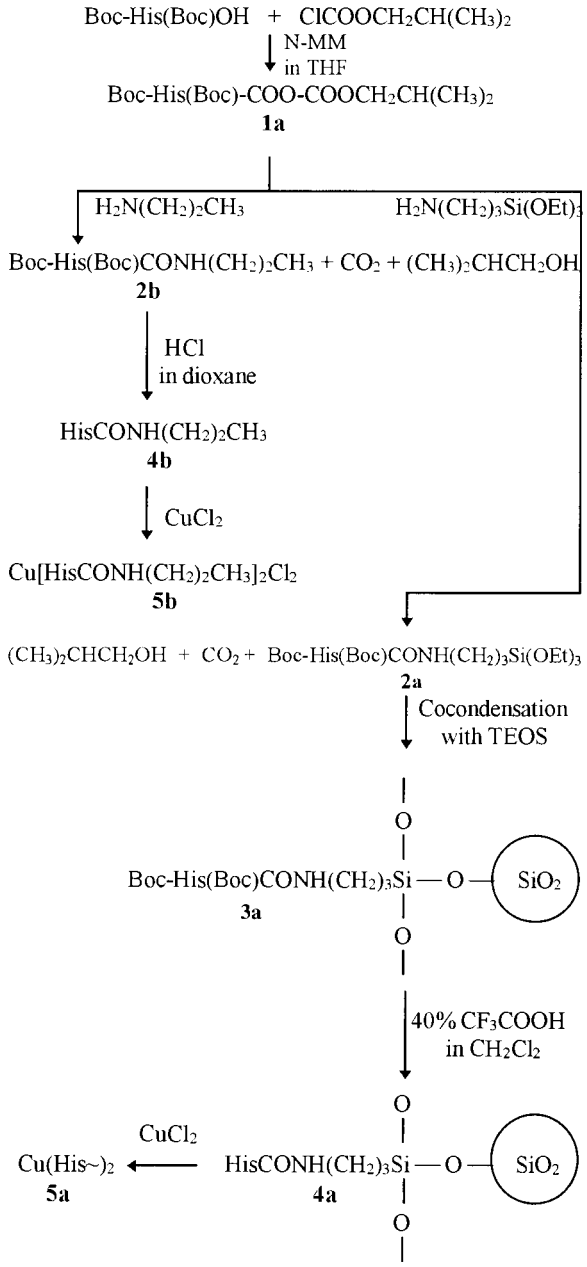
Scheme 1. Synthesis of Cu^{II}(histidine)$_2$ Complexes 5a and 5b



tert-butylcatechol. An internal standard (such as acetophenone) was added to the solution, and the mixture was stirred at room temperature. Aliquots were removed at appropriate time intervals for GC analysis. Blank experiments showed that without catalyst the transformation of DTBC to DTBQ does not take place.

Results and Discussion

The overall reactions for the preparation of the catalysts are outlined in Scheme 1.

The Boc−His(Boc)−CO−NH$(CH_2)_3$Si$(OEt)_3$ monomer **2a** was prepared by reaction of Boc−His(Boc)OH with isobutylchloroformate in the presence of *N*-methylmorpholine (N-MM); the resulting active intermediate **1a** attaches to (3-aminoprolyl)-triethoxysilane. Hydrolysis and co-condensation of the reaction mixture with $Si(OEt)_4$ in a biphasic THF−H_2O system, according to the sol−gel procedure,[5] leads to the insoluble polymer **3a.** After acid deprotection of the Boc groups of His, the [His−$CONH(CH_2)_3SiO_{3/2}]_n$•$xSiO_2$ polymer **4a** was obtained, which exhibits a specific surface area of 430 m^2 g^{-1}.

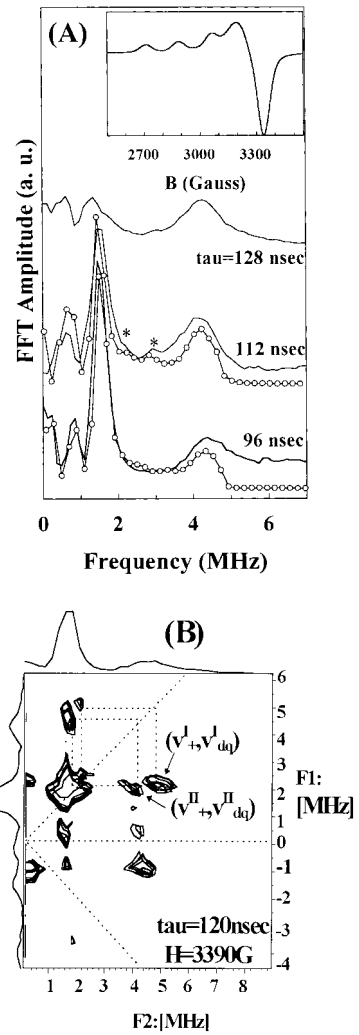


Figure 1. (A) Stimulated FT-ESEEM spectra of **5a** recorded at the indicated τ values. Dashed lines: simulated spectra calculated by using the parameters listed in Table 1. Inset: experimental CW EPR spectrum of complex **5a**. (B) 2D-HYSCORE spectrum (contour plots) of **5a** recorded at $\tau = 120$ ns. The assignment of the main peaks is indicated. Conditions: $H = 3390$ G; $t_{\pi/2} = 16$ ns, $t_\pi = 32$ ns; $T = 15$ K; microwave frequency = 9.62 GHz.

To prepare a complex of **4a** with copper(II), the pH of the **4a** is adjusted to ~7 (material in suspension in distilled water) and an excess of $CuCl_2$ is added. The resulting blue copper(II) polymer **5a** has a ratio of copper(II) to His residue equal to 1:2, and it exhibits a higher specific surface area (650 m^2 g^{-1}) than the **4a** (430 m^2 g^{-1}).

After the 1:1 attachment of the active intermediate **1a** to *n*-propylamine, Scheme 1, the pseudo-peptide Boc−His(Boc)-$CONH(CH_2)CH_3$ (**2b**) is formed. Acid deprotection of the Boc groups of His results in $HisCONH(CH_2)_2CH_3$ (**4b**), and its reaction with $CuCl_2$ in MeOH gives $Cu[HisCONH(CH_2)_2CH_3]_2$-$Cl_2$ (**5b)** complex.

The diffuse-reflectance spectrum of the blue Cu(His~)$_2$ complex **5a** is characterized by an absorption band around 15 600 cm^{-1}. The continuous wave EPR spectrum (inset Figure 1A) is characteristic of a single mononuclear Cu^{II} center with hyperfine splitting ($g_{||} = 2.26$, $g_\perp = 2.063$, and $A_{||} = 174$ G). No superhyperfine couplings from coordinating nuclei were resolved.

The three-pulse stimulated-ESEEM spectrum of **5a** in Figure 1A contains low-frequency components at 0.7 MHz and 1.7 MHz and a broad feature at 4.9 MHz. For a single ^{14}N nucleus

Table 1. Hyperfine and Nuclear Quadrupole Interaction Constants of the $Cu^{II}(His\sim)_2$ Complex **5a**

	N_I	N_{II}
(A_x, A_y, A_z) (MHz)	(1.8, 1.8, 2.6)	(1.8, 1.6, 2.4)
K (MHz)	0.54	0.38
η	0.11	0.84
Euler angles		
A vs **g** (α, β, γ)	(0°, 70°, 0°)	(0°, 0°, 0°)
Q vs **g** (u, v, w)	(0°, 15°, 0°)	(0°, 25°, 0°)

($I = 1$), in the case of exact cancellation, i.e., when the isotropic hyperfine coupling A_{iso} is close to $2\nu_I$, the ESEEM spectrum typically contains three sharp low-frequency lines at frequencies $\nu_\pm = 3K(1 \pm \eta)$ and $\nu_0 = 2K\eta$, where $K = e^2Qq_{zz}/4h$ is the quadrupole coupling constant and η is the asymmetry parameter;[6] in addition a broad double-quantum, $\Delta m_I = 2$, transition occurs at higher frequencies $\nu_{dq} \approx 2[(\nu_I + A_{iso}/2)^2 + K^2(3 + \eta^2)]^{1/2}$. In the corresponding 2D-HYSCORE spectrum, which provides correlations between frequencies originating from different M_S manifolds,[7a,b] cross-peaks at (ν_0, ν_{dq}), (ν_-, ν_{dq}), and (ν_+, ν_{dq}) are usually expected.[7a,b] Typically the cross-peak at (ν_+, ν_{dq}) is easily resolved in experimental HYSCORE spectra, while the other two are much weaker.[7b] The 2D-HYSCORE spectrum of **5a** in Figure 1B exhibits intense cross-peaks at (4.7 MHz, 1.7 MHz) and (3.8 MHz, 1.6 MHz) and a weaker one at (3.8 MHz, 0.7 MHz). The two strong features at (4.7 MHz, 1.7 MHz) and (3.8 MHz, 1.6 MHz) are assigned to two distinct (ν_+, ν_{dq}) cross-peaks. This observation allows an unambiguous assignment of the 4.7 and 3.8 MHz frequencies to *two* double-quantum frequencies ν_{dq} and the 1.7 MHz and 1.6 MHz frequencies to the corresponding ν_+ transitions. These features originate from two ^{14}N nuclei coupled with the electron spin. Based on these assignments, numerical simulations of the stimulated ESEEM spectra (Figure 1A, dashed line) verify this assignment and allow a refinement of the coupling parameters, which are listed in Table 1. Noticeably the lines marked by (*) are "combination lines", and their observation proves that both ^{14}N are coupled to the same copper(II) complex. In summary the ESEEM data show that in **5a** the copper(II) spin is coupled to two ^{14}N nuclei with distinct coupling parameters. We should notice that these two ^{14}N nuclear couplings give rise to two overlapping ESEEM spectra, as can be easily seen from the projection of the HYSCORE spectrum on the F2 frequency axis, Figure 1B top trace. Although the HYSCORE spectrum allows a straightforward resolution and assignment of the overlapping features, this is not as easily achievable based on the 1D-ESEEM spectra, where numerical simulation is necessary.

Comparison of the nuclear quadrupole interaction (NQI) parameters K and η listed in Table 1 with (NQI) values from the literature[10] allows us to discuss the identity of the two interacting nitrogens, $N_{I,II}$. The K, η values for N_{II} are close to those reported for the amino-nitrogen of *N*-acetylhistidine coordinated to copper(II). These are typical for the remote, noncoordinated, nitrogen from imidazole coordinated to copper-(II).[10] The parameters for N_I are close to those reported for 1-methylimidazole coordinated to copper(II).[10] The K, η values for N_I are not often encountered in copper−imidazole systems. Based on the Towens-Dailey model, Peisach and co-workers have shown that in copper−imidazole complexes structural constraints and/or hydrogen bonding can modulate the K, η values.[10b] This in turn reflects modifications of the ^{14}N lone pair population and/or the orientation of the quadrupolar tensor in the imidazole molecular frame.[10] In conclusion, on the basis of the analysis of the K, η values, we consider that in complex **5a** the coordination sphere of copper(II) contains two inequivalent imidazoles. It is tempting to investigate whether this inequivalence is important or not for the observed catalytic activity of **5a**.

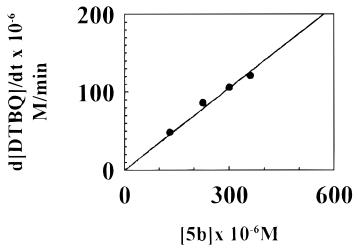


Figure 2. Dependence of DTBQ formation on the initial concentration of **5b**.

Table 2. Efficiency of $Cu^{II}(histidine)_2$ Complexes **5a** and **5b** in Oxidation Catalysis

catalyst	type of catalyst	molar ratio of catalyst:base:substrate	turnover [h-1]	yield of DTBQ [%]	time [h]
5b	homogeneous	0.3:3:200	28	18	48
5a	heterogeneous	0.3:3:200	28	13	48
5a	heterogeneous	0.3:30:200	49	16	48

The ability of complex **5a** to catalyze the oxidation of 3,5-di-*tert*-butylcatechol (DTBC) to 3,5-di-*tert*-butylquinone (DTBQ), acting as heterogeneous catalyst, was determined, and the results were compared with those found for the $Cu[HisCONH(CH_2)_2CH_3]_2Cl_2$ (**5b**) complex, when used as homogeneous catalyst. Both catalysts, **5a** and **5b**, were tested in a real catalytic reaction with a ratio of catalyst to DTBC equal to 0.3:200. More particularly, a solution of DTBC in MeOH was oxidized with molecular oxygen to DTBQ by a solution of **5b** containing 10 equiv of triethylamine (catalyst:base:substrate = 0.3:3:200). The initial rates of DTBQ formation were determined from the maximum slopes of the absorbance versus time plots; these rates were found to be first order with respect to the initial concentration of **5b** (Figure 2).

The DTBQ formation showed a turnover rate of 28 h^{-1} with a yield of 18% (Table 2). To test the catecholase activity of the heterogeneous catalyst **5a**, into a solution of DTBC was added 2.4 mg of $Cu(His\sim)_2$ **5a**, instead of a solution of **5b**. A kinetic study showed turnovers of 28 and 49 h^{-1} when the molar ratio of catalyst to base was 1:10 and 1:100, respectively; in both cases, the yield was almost the same (Table 2). The homogeneous catalyst **5b**, which is a $Cu^{II}(histidine)_2$ complex, shows a remarkable yield of DTBQ formation and an unusually long reaction time. Comparing the efficiency of the homogeneous catalyst **5b** with that of the heterogeneous catalyst **5a**, it is noticed that **5a** decreases slightly the yield of DTBQ formation, but it improves considerably the turnover and mostly remains intact after repeated uses (five times) without changes in its catalytic properties.

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Until now, numerous studies have been performed on the aromatic hydroxylation of an endogenous xylyl substrate by dinuclear copper(I) macrocyclic complexes, which bear the bridging *m*-xylyl moiety.[11] This was mainly prompted by the fact that a μ-peroxo copper(I) center is formed during the oxidation process at the dinuclear copper sites of tyrosinase.[12] Pertinent dinuclear copper(II) complexes have been demonstrated to exist, but only at low temperatures (−60 to −80 °C).[11a,g,13] Studies on the oxidation of substrates by copper(II) at room temperature showed that the initial rates for stoichiometric copper(I)−dioxygen oxidations were 5−100 times greater than for the corresponding copper(II) oxidations.[14a,b] The above data suggest that copper(II) oxidation is an important step of the catalytic cycle, and when copper(II) does not catalyze the oxidation, then the overall reaction is not catalytic in nature.[14a]

There are a few examples of dicopper(II) complexes that exhibit catecholase activity to some extent, but their initial rates (varying from 0 to $\sim 4 \times 10^{-6}$ M min^{-1})[14] and yields (DTBQ formation < 1% or no indication)[14a,c−e] are clearly inferior to those of the present material. Copper(II) complexes with nitrogen-containing tripodal ligands, as models for the protein backbone of tyrosinase, usually show low catalytic activity (varying from 0.0005 to 0.5 μmol substrate per mg catalyst per min, that is, turnover rates from 0.0003 to 0.3 min^{-1}) with only one exception.[15a,b] In the latter case,[15b] a high initial rate for the oxidation of 3,5-di-*tert*-butylcatechol was reported (85 μmol substrate per mg catalyst per min, namely, turnover rate of 50 min^{-1}), but with no further information about the reaction time and the total yield. Previously Cu(II) mononuclear complexes were reported to have catalytic activity for DTBQ formation with a ratio of catalyst to DTBC from 2:1 to 1:5.[15c] However, it is clear that this is a simple stoichiometric reaction rather than a catalytic one. In contrast, our **5a** and **5b** complexes show clearly a catalytic activity for DTBQ formation evidenced by the 0.3:200 ratio of catalyst to substrate.

To our knowledge, the present system is the first example of a supported Cu^{II}(amino acid) complex in which, first, the amino acid is bonded covalently via a peptide bond with the silica matrix and second the silica matrix was made in situ, giving the opportunity to engineer some microstructural properties, such as specific surface area of the material. The application of this procedure, by using a simple His as anchoring amino acid on an inorganic support, leads to a hybrid organic−inorganic material. This material is stable and easy to prepare and shows significant catalytic activity for the DTBC oxidation. In evaluating its catalytic properties, we point out that it is highly recyclable and exhibits significant catecholase activity in the presence of dioxygen, with turnovers ranging from 28 to 49 h^{-1} and considerable yields (13%−16%).

The experimental conditions that were used led to mononuclear copper complexes with two histidine molecules per copper(II) atom according to the 2D-ESEEM data. The above technique was used as a powerful tool for the detailed characterization of the microenvironment of copper in the new material, and this is important from the spectroscopic point of view.

In conclusion our work presents a new general approach for the chemical modeling of the active site of copper−proteins. It would be interesting to examine whether this approach could be applied to other amino acids or peptides in order to mimic the active sites of other metalloenzymes.

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