SUPPLEMENTARY INFORMATION

A dinuclear biomimetic Cu complex derived from L-histidine: Synthesis and stereoselective oxidations

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Derivation of the kinetic equation for a catalytic reaction requiring the binding of two substrate molecules.

The catalytic oxidations of norepinephrine enantiomers by \([\text{Cu}_2(\text{EHI})]^{4+}\) show a substrate sigmoidal dependence suggesting the binding of two substrate molecules to the catalyst in order to observe catalysis. The reaction scheme describing the catalytic behavior is as follow:

\[
C + 2S \overset{\beta_2}{\longrightarrow} \text{CS}_2 \overset{k_{\text{cat}}}{\longrightarrow} \text{C} + \text{S} + \text{P}
\]

where C is the catalyst, S is norepinephrine, \(\text{CS}_2\) is the catalyst bound to two substrate molecules and P is the product of the reaction.

Assuming that:

a) The binding step I fast with respect to the turnover cycle rate (as shown by the fast binding observed in the binding studies with \([\text{Cu}_2(\text{EHI})]^{4+}\) and considering the slow reaction rates, i.e. moderate \(k_{\text{cat}}\) values)

b) The binding of two substrate molecules is needed to perform the reaction (i.e., the CS species is catalytically not efficient)
c) Only a minor fraction of the complex with bound only one substrate molecule accumulates during turnover (i.e., $[CS]<<[C]+[CS_2]$)

According to point a, the binding of the two substrate molecules occurs as a pre-equilibrium, allowing the use of the binding constant $\beta_2$ (equation 1), together with the mass balance on the catalyst (equation 2), to obtain the species concentration during turnover

$$C + 2S \xrightarrow{\beta_2} CS_2 \quad \beta_2 = \frac{[CS_2]}{[C_0] \cdot [S]^2} \quad (1)$$

$$[C_0] = [C] + [CS_2] \quad (2)$$

where $[C_0]$ is the total (free plus substrate bound) concentrations of the catalyst

$[CS_2]$ can be obtained from equation 1, $[CS_2] = \beta_2 \cdot [C] \cdot [S]^2$

The substitution of $[CS_2]$ in equation 2 gives the free (not bound) catalyst concentration:

$$[C] = \frac{[C_0]}{1 + \beta_2 \cdot [S]^2} \quad (3)$$

And then that of the $CS_2$ species:

$$[CS_2] = \frac{[C_0] \cdot \beta_2 \cdot [S]^2}{1 + \beta_2 \cdot [S]^2} \quad (4)$$

The reaction rate depends on $[CS_2]$ through equation 5:

$$r = k_{cat} \cdot [CS_2] \quad (5)$$

The rate equation is obtained by combining equations 4 and 5:

$$r = \frac{k_{cat} \cdot [C_0] \cdot \beta_2 \cdot [S]^2}{1 + \beta_2 \cdot [S]^2} \quad (6)$$

and

$$\frac{r}{[C_0]} = \frac{k_{cat} \cdot \beta_2 \cdot [S]^2}{1 + \beta_2 \cdot [S]^2} = \frac{k_{cat} \cdot [S]^2}{1/\beta_2 + [S]^2} = \frac{k_{cat} \cdot [S]^2}{K' + [S]^2} \quad (7)$$

where $K' = 1/\beta_2$
Derivation of the kinetic equation to interpret the monomeric-dimeric equilibrium of [Cu$_2$(EHI)]$^{4+}$.

In order to model this peculiar behavior, the kinetic equations were appropriately derived with the assumption that, in substrate-saturating conditions, the oxidation rate depends only on complex concentration. We also assume that the complex exists in two forms in dynamic equilibrium, a monomeric and a dimeric species:

$$2C \rightleftharpoons C_2 \quad \text{ruled by } K_b = [C_2]/[C]^2$$

Oxidation rate depends from both [C] and [C$_2$]

$$r = k_1[C] + k_2[C_2]$$

Considering the mass equation:

$$[C_0] = [C] + 2K_b[C]^2$$

appropriate substitution leads to the final equation, used for the interpolation:

$$r = k_1 \left( -1 + \frac{1 + 8 \times K_b \times [C_0]}{4K_b} \right) + k_2K_b \left( -1 + \frac{1 + 8 \times K_b \times [C_0]}{4K_b} \right)^2$$

**Figure 1S.** (a) UV-Vis spectra of [Cu$_2$(EHI)]$^{4+}$ in methanol, 0.1 mM. (b) Magnification of the low energy region.
**Figure 2S.** (a) Family of UV-Vis spectra taken upon addition of a concentrated solution of NaN$_3$ to [Cu$_2$EHI]$^{4+}$ in 9:1 methanol/acetonitrile (v/v) solution. Solid black lines: initial and final spectra of the titration, corresponding to [Cu$_2$EHI]$^{4+}$ and the mixture of [Cu$_2$EHI(N$_3$)]$^{3+}$ and [Cu$_2$EHI(N$_3$)$_2$]$^{2+}$ species, respectively. (b) Distribution diagram (concentration vs. equiv. of added NaN$_3$) of the species, calculated for log $K_{b1} = 4.61$, and log $K_{b2} = 3.59$, according to reactions (1) and (2), respectively.

[Cu$_2$EHI]$^{4+}$ + $N_3^-$ ⇌ [Cu$_2$EHI(N$_3$)]$^{3+}$ \hspace{1cm} (1)

[Cu$_2$EHI(N$_3$)]$^{3+}$ + $N_3^-$ ⇌ [Cu$_2$EHI(N$_3$)$_2$]$^{2+}$ \hspace{1cm} (2)

Solid and dotted black lines: free [Cu$_2$EHI]$^{4+}$ and [Cu$_2$EHI(N$_3$)]$^{3+}$, respectively; dashed black line: [Cu$_2$EHI(N$_3$)$_2$]$^{2+}$. The graph shows the experimental profile of absorbance vs. equiv. NaN$_3$ at 385 nm (diamonds) and the fitted curve (dashed line).
Figure 3S. (a) $^1$H-NMR spectral variation of a solution of [Cu$_2$(EHI)](ClO$_4$)$_4$ (2.2 mM) in deuterated methanol (MeOD) upon increasing the temperature (trace 1: -15 °C; 2: 0 °C; 3: 15 °C; 4: 35 °C); (b) $^1$H-NMR spectral variation of a solution of [Cu$_2$(EHI)](ClO$_4$)$_4$ (1.98 mM) in MeOD/deuterated acetate buffer (50 mM, pH 5.1) 10:1 (v/v) upon increasing the temperature (trace 1: -15 °C; 2: 0 °C; 3: 15 °C; 4: 25 °C; 5: 35 °C).
Figure 4S. Effect of substrate concentration on the initial rate of oxidation of L-/D-Dopa (a), L-/D-DopaOMe (b), and R-/S-norepinephrine (c) by [Cu₂EHI](ClO₄)₄ (1 μM) in a 10:1 (v/v) mixture of methanol/aqueous acetate buffer (50 mM) at pH=5.1.
Figure 5S. Effect of substrate concentration on the initial rate of oxidation of L-/D-Dopa (a), L-/D-DopaOMe (b), and R-/S-norepinephrine (c) by [Cu_{2}EHI](ClO_{4})_{4} (5 μM) in a 10:1 (v/v) mixture of methanol/aqueous acetate buffer (50 mM) at pH=5.1.
Figure 6S. (Left) $^1$H NMR spectrum in acetone d-6 (δ values, ppm) of the crude product mixture of hydroxylation reaction of the TBA salt of L-tyrosine. (Right) Expansion of the aromatic region of the spectrum, unreacted phenol signals are indicated with arrows.

Figure 7S. (Left) $^1$H NMR spectrum in acetone d-6 (δ values, ppm) of the main product from the hydroxylation reaction of the TBA salt of L-tyrosine. (Right) Expansion of the aromatic region of the spectrum.
Figure 8S. GC-MS chromatogram of product mixture of the hydroxylation reaction of the TBA salt of L-tyrosine (upper trace) and fragmentation pattern of the main product (bottom trace), with retention time of 26.25 min.
Figure 9S. UV-Vis spectral changes observed during the oxidation of tetrabutylammonium salts of \(N\)-acetyl-L-tyrosine ethyl ester (left), and \(N\)-acetyl-D-tyrosine ethyl ester (right) by \([\text{Cu}_2(\text{EHI})]^2+/\text{O}_2\).