Dioxygen Binding to a Macrocyclic Dinuclear Copper(I) Monooxygenase Model System. Ambient and High Pressure Kinetics

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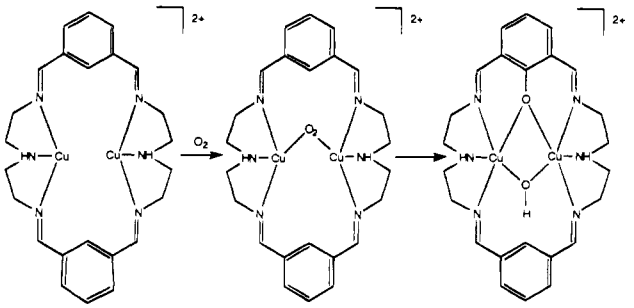
Tyrosinase, a dinuclear copper protein, is a monoxygenase which activates dioxygen for the ortho hydroxylation of monophenols (tyrosine) and further oxidizes the *o*-diphenol to an *o*-quinone.[1,2] Efforts to functionally model this protein and the related dioxygen transport protein hemocyanin led to the development of a series of mononuclear and dinuclear copper model complexes.[3−6] Reaction of dioxygen with the dinuclear Cu(I) complex $[Cu_2(R-XYL-H)]^{2+}$ (**1**), thoroughly investigated by Karlin and co-workers,[7−14] led to specific hydroxylation of the xylyl ligand yielding a phenolate bridged Cu(II) complex $[Cu_2(R-XYL-O-)OH]^{2+}$ (**2**). Substitution of the pyridines of the ligand by other nitrogen donors, e.g. pyrazole,[15,16] or changing the ethyl pyridine to a methyl pyridine group,[17] completely suppressed the hydroxylation reaction. Instead a hydroxo-bridged dinuclear Cu(II) complex was obtained as product.[15] In contrast, dinuclear bis(imine) Cu(I) complexes with different nitrogen donor groups showed hydroxylation of the xylyl ligand in most cases studied.[18−22]

Detailed kinetic studies on the reversible binding of dioxygen and the subsequent hydroxylation reaction are required to provide insight into the intimate mechanisms of these reactions. Such studies on these and related systems are limited.[9,12,14,23,24] It was found that complex **1** in dichloromethane at low temperature rapidly and reversibly binds dioxygen followed by a slower hydroxylation step.[12] The formation of a peroxo complex could be observed in the UV−vis spectra. These reactions are expected to involve significant conformational changes such that the reported activation parameters ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) are composite quantities.[12]

We have shown that the application of high pressure kinetic techniques along with the construction of volume profiles can significantly assist the elucidation of the intimate mechanism.[25−30] In an effort to improve our understanding of the various reactions of dioxygen with the Cu(I) complexes mentioned above, we have initiated a series of detailed kinetic studies including the application of rapid-scan and high pressure kinetic techniques. For our first studies we selected the Cu(I) complex **3**, which was originally synthesized and investigated by Menif et al.[22] **3** reacts with dioxygen to give the adduct **4**, which has so far not been characterized, followed by decomposition to the hydroxylated product **5**. During the reaction of **3** with dioxygen in dichloromethane, approximately 75% of the product showed hydroxylation of one benzene ring of the ligand; the other 25% was assigned to simple hydroxy-bridged Cu(II) species.[22] Similar findings were reported for complex **1** by Karlin et al.[12] We studied the reaction of **3** with dioxygen in methanol, which proved to be a good reaction medium for hydroxylation reactions.[16,18] The reaction was studied under pseudo-first-order conditions with dioxygen kept in excess over **3**. UV−vis repetitive scan spectra were recorded on a Durrum



3 **4** **5**

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D110 stopped-flow equipped with a J&M detector connected to a TIDAS 16-416 spectrophotometer. The reaction showed isosbestic points at 350, 395 and 545 nm (Figure S1). Absorbance-time traces at different wavelengths showed good first order behavior. A linear dependence of k_{obs} on the dioxygen concentration was observed at different temperatures (Figure S2). There is no evidence for a back-reaction as indicated by the absence of an intercept in this plot. The complete rate law therefore is ($k_{obs} = k[O_2]$):

$$-d[\mathbf{3}]/dt = k[\mathbf{3}][O_2]$$

with a second order rate constant k of 124 ± 2 M^{-1} s^{-1} at 25 °C. The activation parameters for the second order rate constant are $\Delta H^{\ddagger} = 32 \pm 2$ kJ/mol and $\Delta S^{\ddagger} = -146 \pm 8$ J/(mol K). The effect of pressure was measured on a homemade high-pressure stopped-flow instrument,[31] and the results are shown in Figure S3. An increase in pressure caused an increase in the values of k_{obs}. The obtained activation volume $\Delta V^{\ddagger} = -21 \pm 1$ cm^3/mol.

The kinetic data are in line with three possible mechanisms. (a) The rate determining step could be the reaction of dioxygen with one of the two Cu(I) ions. This would mean a coordinated solvent molecule, in our case acetonitrile (from the complex preparation), must be substituted by dioxygen. Our $\Delta H^{\ddagger}$ value of 32 ± 2 kJ/mol is the same as was found for the substitution of acetonitrile by dioxygen for a mononuclear copper(I) complex.[32] However, the reaction is much faster than in our case and is accompanied by a $\Delta S^{\ddagger}$ value of 14 ± 18 J/(mol K), whereas we find $\Delta S^{\ddagger} = -146 \pm 8$ J/(mol K). In addition, the very negative $\Delta V^{\ddagger}$ of −21 cm^3/mol is not typical for a ligand substitution process.[25,26] By way of comparison a value of −4.7 ± 0.3 cm^3/mol was found for the substitution controlled binding of dioxygen to a macrocyclic Co(II) complex.[29] (b) Another possibility is a fast preequilibrium that leads to the dioxygen adduct **4** (K) followed by the rate determining hydroxylation of the complex (k). We do not observe any spectral buildup of a dioxygen adduct immediately after mixing **3** and O_2 in our rapid scan experiment (Figure S1), which means that if such a preequilibrium is present it must lie almost totally to the left side, i.e. mainly **3**. In that case $k_{obs} = kK[O_2]$. Such a behavior was observed for complex **1** at room temperature, whereas at low temperature the peroxo complex was quite stable.[12] For the reaction of the dioxygen adduct of **1** to **2**, $\Delta H^{\ddagger} = 50 \pm 1$ kJ/mol and $\Delta S^{\ddagger} = -35 \pm 2$ J/(mol K) were found, which differ significantly from our values. (c) The formation of the dioxygen adduct **4** could be the rate-determining step and the subsequent reaction to the hydroxo-bridged complex **5** could be fast. This mechanism was suggested by Menif et al.[22] Comparing the activation parameters for **1** and **3** for that reaction shows that $\Delta S^{\ddagger} = -146$ J/(mol K) for both reactions, whereas $\Delta H^{\ddagger} = 8.2 \pm 0.1$ kJ/mol for **1** compared to our value of 32 ± 2 kJ/mol. The $\Delta V^{\ddagger}$ value of −21 ± 1 cm^3/mol found in the present study is indeed very close to the average value of −22 ± 2 cm^3/mol reported recently for the oxidation of Cu(I)(phen)$_2$ by dioxygen.[33] In the latter study it was concluded that the significantly negative volume of activation mainly arises from the large volume collapse associated with the formation of the intermediate (phen)$_2$Cu^{I}−O_2 species.[33] Formation of adduct **4** involves the complete binding of oxygen and therefore exhibits a similar pressure dependence to that found for the Cu^{I}(phen)$_2$ system. Additional support for this mechanism comes from the results for the reaction of an Ir(I) complex with dioxygen in MeOH.[34] Here the formation of a dioxygen adduct is characterized by $\Delta H^{\ddagger} = 26.8 \pm 2.1$ kJ/mol, $\Delta S^{\ddagger} = -192 \pm 8$ J/(mol K), and $\Delta V^{\ddagger} = -31.1 \pm 1.7$ cm^3/mol, which are close to our data. The strongly negative $\Delta S^{\ddagger}$ and $\Delta V^{\ddagger}$ values support the concept of a highly structured transition state, as a result of the highly reactive and easily oxidizable cuprous species. The very negative volume of activation must be a strong indication of Cu−O_2 bond formation that is accompanied by electron transfer to produce the Cu(II)−O_2−Cu(II) peroxo intermediate. The formal oxidation of Cu(I) to Cu(II) and reduction of O_2 to O_2^{2-} are expected to be accompanied by a significant volume collapse, partly due to intrinsic and solvational volume changes.[25] Similar effects were reported for the binding of aliphatic radicals to Co(II) and Cr(II) complexes, which is also accompanied by a formal electron transfer (M^{II}−R → M^{III}−R^-) and a significant volume collapse.[27,28] Such oxidative addition reactions in general exhibit significantly negative volumes of activation.[30,34,35]

Although the available kinetic data do not allow an unequivocal assignment of the mechanism, we conclude from the arguments outlined above that mechanism c is presently the most likely possibility. If this is true, an explanation for the different behavior of **1** and **3** with dioxygen at room temperature could be the different solvents used in the kinetic experiments. It was shown for copper imine complexes that protons facilitate hydroxylation and may therefore also increase the rate of this reaction.[36] Addition of water or acetic acid to a solution of **3** did not increase the rate of the reaction, which would be expected if the hydroxylation reaction is the rate-determining step. On the other hand, if the acetonitrile concentration in solution is increased 20-fold, the observed rate constant decreases by a factor of 2.5. This clearly suggests that the formation of the adduct **4** must be the rate determining step.

The results of this study clearly demonstrate the potential of such kinetic studies to improve our insight into the detailed mechanism of these oxygenation reactions. The data now available for two types of dinuclear Cu(I) complexes are encouraging and motivate further studies on a series of imine and non-imine complexes in different solvents under ambient and high pressure conditions.

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Supplementary Material Available: Figure S1, repetitive scan spectra of the reaction of **3** with dioxygen, Figure S2, plots of k_{obs} vs $[O_2]$ as a function of temperature, and Figure S3, plot of ln k vs pressure (3 pages). Ordering information is given on any current masthead page.

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