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VIP Model Heme Complexes Very Important Paper

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Binding of O_2 and CO to Metal Porphyrin Anions in the Gas Phase**

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Metal porphyrins and their derivatives are the key cofactors in many heme enzymes, which themselves are present in virtually all living organisms. In heme proteins (e.g. cytochrome P450 or hemoglobin), an iron porphyrin complex serves as the prosthetic group and acts as a potent catalyst for oxygen transfer and transport.[1] These enzymes ultimately allow living cells to use otherwise unreactive molecular oxygen directly from the environment. In most such biochemical processes the catalytic cycle starts by binding dioxygen to the ferrous heme group, that is, after the iron porphyrin complex has been reduced from its resting ferric(III) state to an active iron(II) center.[2] It has long been recognized that oxygen binding in heme enzymes occurs in a highly cooperative manner[3] and is crucially modulated by a proximal axial ligand at the Fe^{II} center and its propensity for electron donation or withdrawal. Fourfold-coordinated iron porphyrins (4c-FeP; no axial ligand) are known from spectroscopic experiments to exhibit a triplet ground state ($S=1$).[4] For 5c-FeP, the spin multiplicity is found to be a quintet ($S=2$), with the exact nature of the axial ligand giving rise to subtle but important differences on the reactivity towards dioxygen. As suggested recently,[5] the weakly donating imidazole and histidine ligands (as in hemoglobin or myoglobin, respectively) lead to reversible Fe-O_2 binding, whereas more strongly donating ligands (such as anionic imidazolate) may be responsible for stronger oxygen fixation (as in cytochrome P450).

Towards an improved understanding of the elementary binding processes occurring in model heme complexes, gas-phase experiments—in particular those involving mass-to-charge ratio selected ions—offer the advantage that the oxidation state of the metal center may be controlled,[6] while environmental effects such as counterions or solvent molecules can be rigorously excluded. Along these lines, Crestoni and co-workers[7] have extensively studied the gas-phase ion chemistry of metal porphyrin ions in ion cyclotron resonance (ICR) cells at room temperature. They found no strong ligand effect for the low-pressure association equilibria of Fe^{II} and Fe^{III} heme cations in the systems studied, but were able to derive a thermochemical ladder (i.e. differences in ΔG values) for the binding of various neutral reagents such as pyridine and NO.[8] In another ICR study (over the elevated temperature range 308–342 K), the association reaction of several iron(II) porphyrin cations (with different peripheral substituents) with NO was investigated by Ridge and co-workers.[9] They derived NO binding energies to be in the range of 103.8–120.9 kJ mol^{-1}, that is, with only small variations depending on the peripheral ligand of the porphyrin. Moreover, virtually identical numbers were obtained for singly and doubly charged iron porphyrin cations.

Here we report the first experimental determinations of the binding energies of molecular oxygen and carbon monoxide to model metal porphyrin complex ions in vacuo. For this, the association reactions of deprotonated iron(II) tetrakis(4-sulfonatophenyl)porphyrin tetraanions $[Fe^{II}(tpps)]^{4-}$ (Figure 1) and the manganese(II) tetrakis(4-sulfonatophenyl)porphyrin species $[Mn^{II}(tpps)]^{4-}$ with O_2 and CO were probed over a temperature range of 120 to 210 K in a variable-temperature Penning ion trap (Figure 2).[10]

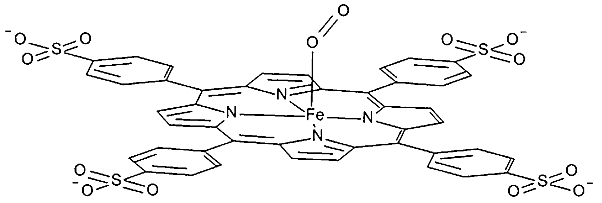

Figure 1. Oxygen complex of $[Fe^{II}(tpps)]^{4-}$. tpps = tetrakis(4-sulfonatophenyl)porphyrin.

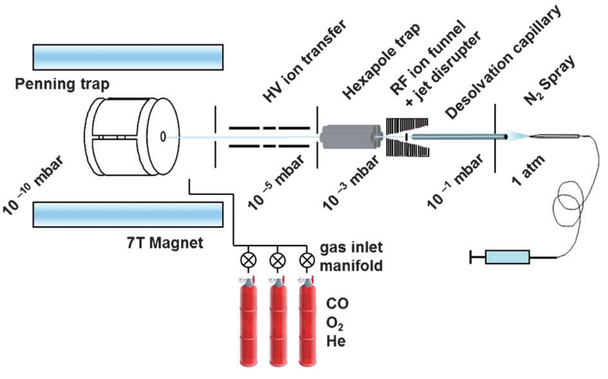

Figure 2. Schematic representation of the experimental setup.

Trapped $[Fe^{II}(tpps)]^{4-}$ and $[Mn^{II}(tpps)]^{4-}$ were allowed to react at low temperatures with O_2 or CO to give the molecular association products. Figure 3 exemplifies this type of reaction for the system $[Fe^{II}(tpps)]^{4-}/O_2$ at $T=123$ K, without any side reaction being observable in the corresponding mass

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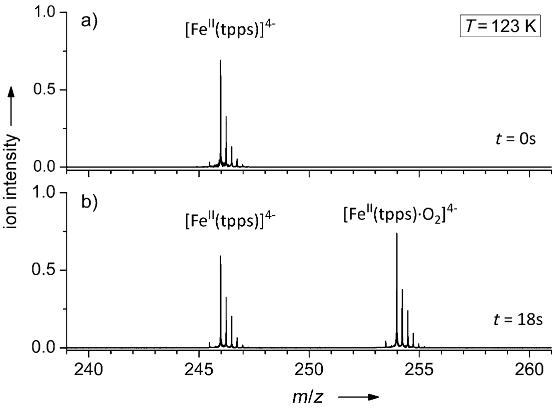

Figure 3. Negative-ion mass spectra of $[\mathrm{Fe^{II}(tpps)}]^{4-}$ a) before and b) after addition of oxygen for 18 s at a partial pressure of 4×10^{-7} mbar and at a temperature of 123 K.

spectra. Similarly, $[\mathrm{Fe^{II}(tpps)}]^{4-}$ already reacts readily with CO at temperatures around 180–210 K, as does $[\mathrm{Mn^{II}(tpps)}]^{4-}$ with O_2.[11]

Kinetic data obtained by evaluating the ion concentration at constant reactant pressure versus reaction time clearly show that the reaction comes to an equilibrium for longer times at sufficiently low temperatures (shown in Figure 4 for the iron/O_2 system) and the reaction can simply be described by Equation (1).

$$[\mathrm{Fe^{II}(tpps)}]^{4-} + \mathrm{O_2} \underset{k_{\mathrm{des}}}{\overset{k_{\mathrm{ad}}}{\rightleftharpoons}} [\mathrm{Fe^{II}(tpps)\cdot O_2}]^{4-} \tag{1}$$

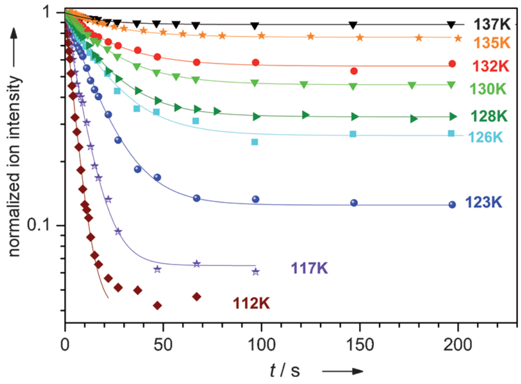

Figure 4. Kinetics for the association of oxygen to trapped $[\mathrm{Fe^{II}(tpps)}]^{4-}$ (O_2 partial pressure 4×10^{-7} mbar) as a function of temperature. Solid lines are fits to the experimental data using the underlying association and dissociation rate equations. Equilibrium constants K_p were obtained by parameterizing the stationary/equilibrium signals observed at long times.

The reversibility of the reaction could be clearly demonstrated in control experiments: upon turning off the O_2 or CO supply after the reaction, the product complex loses the diatomic molecule to return the reactant metal porphyrin ion (see Figure S1/S2 in the Supporting Information). Also included in Figure 4 (as solid lines) are fits of appropriate rate equations using a simple two-step mechanism comprising (radiative) association and (activated) dissociation steps.[10b] The values obtained for the rate coefficients are also summarized in the Supporting Information.

This then allows the equilibrium constants K_p to be determined from the ratio of the rate coefficients k_{ad}/k_{des} and known reactant gas pressure. Figure 5 shows such results as a function of temperature for the three reaction systems studied. From such a van't Hoff plot of the equilibrium constants ($-\ln K_p$ versus inverse temperature) and a linear fit to the data one can directly deduce the reaction enthalpies and entropies for the reaction of O_2 or CO to the metal porphyrin (Table 1). Note that the negative of the reaction enthalpy can be equated to the binding energy of the respective diatomic molecule. Our results show that molecular oxygen is bound to $[\mathrm{Fe^{II}(tpps)}]^{4-}$ by 40.8 kJ mol^{-1}. The corresponding carbon monoxide binding energy is considerably larger (66.3 kJ mol^{-1}). At 67.4 kJ mol^{-1}, dioxygen is found to bind significantly more strongly to the $\mathrm{Mn^{II}}$ center than to the iron porphyrin.

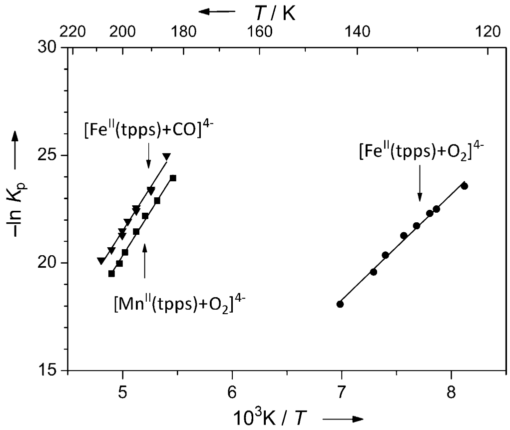

Figure 5. Van't Hoff plot of experimentally determined equilibrium constants K_p for the gas-phase association reactions indicated. Reaction enthalpies were derived from the slope of linear fits to the data (solid lines).

Table 1: Measured reaction enthalpies ΔH_r and entropies ΔS_r for the association reaction to form the respective O_2/CO complex.

	$[\mathrm{Fe^{II}(tpps)\cdot O_2}]^{4-}$	$[\mathrm{Fe^{II}(tpps)\cdot CO}]^{4-}$	$[\mathrm{Mn^{II}(tpps)\cdot O_2}]^{4-}$
ΔH_r [kJ mol^{-1}][a]	-40.8 ± 1.4	-66.3 ± 2.6	-67.4 ± 2.2
ΔS_r [J K^{-1} mol^{-1}]	-134 ± 10	-153 ± 13	-168 ± 11

[a] Errors are estimated from possible systematic errors in temperature (±5 K) and pressure (factor of 2) as well as from the statistical fit errors.

It is interesting to compare our results with literature values from quantum-chemical computations for model iron and manganese porphyrins as well as to relevant studies on heme proteins in the condensed phase (Table 2). It has been pointed out in a number of theoretical studies that the relative binding energies of O_2, CO, and NO to ferrous heme[12] increase in the order $E_b(O_2) < E_b(CO) < E_b(NO)$.[13] However, a more quantitative theoretical description still appears challenging. In a recent study Radon and Pierloot[14] con-

Table 2: Binding energies of O_2 and CO to several model complexes from computations of Fe^{II} and Mn^{II} porphyrins and from solution-phase studies on heme proteins.

Model system	E_b [kJ mol^{-1}]	Reference
Fe porphyrin + O_2		
$[Fe(tpps)]^{4-}$	40.8 ± 1.3	this work
FeP	37.7	DFT, Rovira et al.(1997)[13]
FeP	54.0	DFT, Estrin et al. (2008)[16]
FeP/Fe(tpp)	56.0/75.3	DFT, Zhang et al. (2007)[17]
FeP/$[FePPIX]^{2-}$	55.2/60.2	DFT, Witko et al. (2006)[18]
$[Fe(heme)(his)]^{2-}$	89.9	DFT, Witko et al. (2007)[19]
FeP	32.8	DFT, Sun et al. (2009)[20]
FeP	−5.4–67.4	DFT/Radon et al. (2008)[14]
FeP	44.8	CASPT2, Radon et al. (2008)[14]
human HbA	55.2 ± 1.7	Gaud et al. (1974)[21]
myoglobin	75.7 ± 1.7	Keyes et al. (1971)[22]
Hb	43.1 ± 4.6	Johnson et al. (1992)[23]
Fe{Piv_3(5ClImP)Por}	68.2 ± 4.6	Collman et al. (1978)[24]
Fe porphyrin + CO		
$[Fe(tpps)]^{4-}$	66.3 ± 2.6	this work
FeP	108.8	DFT, Rovira et al. (1997)[13]
FeP	139.3	DFT, Estrin et al. (2008)[16]
FeP	−4.2–110.9	DFT, Radon et al. (2008)[14]
FeP	66.9	CASPT2, Radon et al. (2008)[14]
$[Fe(heme)(his)]^{2-}$	156.9	DFT, Witko et al. (2007)[19]
human HbA	74.1 ± 1.7	Gaud et al. (1974)[21]
myoglobin	89.5 ± 1.3	Keyes et al. (1971)[22]
Mn porphyrin + O_2		
$[Mn(tpps)]^{4-}$	67.4 ± 2.2	this work
MnP	48.9	DFT, Witko et al. (2006)[18]

cluded that density functional theory tends to underestimate the binding energy—except when using the BP86 functional. For example, the binding energy of O_2 to 4c-FeP was found to be anywhere between -5.4 kJ mol^{-1} (B3LYP), that is, not bound at all, and 67.4 kJ mol^{-1} for BP86. However, in the same study it was found that a description using methods based on wave functions, including perturbation theory (CASPT2), reproduces the binding energetics as found in solution-phase studies of wild-type myoglobin (a 5c-FeP complex within its protein environment) reasonably well.[15]

Our gas-phase values for the binding energies of O_2 and CO to iron(II) porphyrin ions agree well with the CASPT2 description (Table 2).

Another theoretical study has considered the influence of charge on the binding propensity of iron(II) porphyrin.[18] The oxygen binding energy of the dianionic iron protoporphyrin IX (FePPIX), which carries two deprotonated propionic acids at the porphyrin ring perimeter, was found to increase by about 10% over the neutral iron porphyrin (FeP). In the seminal computations of Rovira et al.[13] it was also concluded that peripheral ligands only have a minor influence on the chemical behavior of the iron center.

In summary, the experimental work described herein gives benchmark thermochemical information for one of the most important biochemical reactions. Such data on the binding energy of dioxygen and carbon monoxide to iron and manganese porphyrins may prove helpful towards a quantitative quantum-chemical description of the spin-forbidden reaction steps involved in the corresponding enzymatic reactions.[25] In particular, it should help to disentangle the intrinsic properties of the metal–organic center from environmental influences often referred to in the literature as the "protein" effect.

Experimental Section

Experiments were performed with a 7T-FT-ICR mass spectrometer[26] (APEX II, Bruker Daltonics) equipped with an electrospray ion source (ESI, Analytica of Branford) and a home-built ion funnel with jet disrupter.[27] Solutions of Mn^{III} meso-tetra(4-sulfonatophenyl)porphin chloride and Fe^{III} meso-tetra(4-sulfonatophenyl)porphin chloride (cat. nos MnT1239 and FeT1239 from Frontier Scientific, Logan, UT, USA), were prepared in pure water at a concentration of ca. 10^{-5} mol L^{-1}. Some experiments also made use of a home-built nanospray source. Negative ions leaving the capillary were efficiently collected in the ion funnel, prestored in a hexapole ion trap, and pulsed into the ICR cell. Trapped ions were then excited/detected by standard ICR techniques. To perform temperature-dependent kinetic experiments of ion–molecule reactions, the Infinity ICR cell was recently modified[10a] to allow the cell to be heated or cooled within a temperature range of $T = 90$ to 420 K.[28] To obtain kinetic data we followed a mass-spectrometric protocol similar to the one recently described.[10a] Briefly, the reactant ions were isolated in the ICR cell and thermalized with helium or argon at a pressure of 10^{-5} mbar for 5 s. After a pump delay, the reactant gases (O_2 or CO) were dosed into the chamber of the ion trap to give a constant partial pressure of typically around 4×10^{-7} mbar by means of a pulsed solenoid valve. The purity of the gas, as monitored online with a residual gas analyzer, was better than 99.5%.

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