Binding of Nitric Oxide to Iron(II) Porphyrins: Radiative Association, Blackbody Infrared Radiative Dissociation, and Gas-Phase Association Equilibrium

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The binding of NO to an Fe(II)porphyrin has received attention recently because of discoveries revealing the role of that interaction in important biological functions.[1,2] In particular it was found that NO is produced endogenously and that the binding of NO to enzyme-bound heme may play a biological regulatory role.[1,2] The NO molecule is typically much more strongly bound to an Fe(II) porphyrin than is O_2 or CO, which complicates examination of the bonding and determination of binding constants since the addition of NO goes to completion at very low NO pressures. This suggests the examination of the NO−iron porphyrin interaction in the gas phase using Fourier transform ion cyclotron resonance mass spectrometric techniques which, in fact, require a low NO pressure. We report here the results of such an examination. In particular, we report the observation of radiative association[3] of NO to metalloporphyrins in the gas phase and deduce estimates of the metalloporphyrin−NO bond strengths from the rates of the observed associations. At ambient temperature the association reactions go to completion, but at higher temperatures the reaction goes to equilibrium. There is little precedent for the observation of associative equilibria under low-pressure conditions where association occurs by radiative stabilization rather than by collisional stabilization. Equilibrium is observed in this case, however, and an estimate of the bond strength can be made from the equilibrium constant. We also report observation of blackbody infrared radiative dissociation (BIRD)[4,5] of a metalloporphyrin−NO complex in the gas phase and deduce an estimate of the bond strength from the rates of that process. NO−metalloporphyrin binding constants and binding energies do not appear to have been previously measured in the gas phase, although metalloporphyrin−NO complexes have been observed in the gas phase,[6] and NO binding energies have been determined theoretically.[7]

Electrospray ionization of iron tetrapyridylporphyrin chloride[8] (FeTPyrPCl)produced an abundant doubly protonated FeTPyrPH$_2^{+2}$ ion and a less abundant singly protonated FeTPyrPH$^+$ ion. The FeTPyrPClH$_2^{2+}$ and FeTPyrPClH$^+$ ions were also observed, but they did not react with NO. The ions were introduced into the ion trap of a Bruker 70e Bioapex Fourier transform ion cyclotron resonance mass spectrometer(FT-ICR-MS).[9] NO was present at low pressure[10] in the vacuum chamber containing the ion trap. Mass spectra taken at various reaction times show that both FeTPyrPH$_2^{+2}$ and FeTPyrPH$^+$ react to form NO adducts. In the gas phase, colliding species can associate only if the collision complex loses energy by a third-body collision or by radiation; otherwise, the complex will dissociate into its constituent species. The rate of radiative association can be determined by extrapolating the pressure dependence of the association process to zero pressure. The rate of ion neutral radiative association depends most sensitively on the binding energy of the association complex and less sensitively other properties of the associating ion neutral complex such as vibrational frequencies. This makes it possible to make good estimates of bond strength from the radiative association rates.[3] The radiative association rates and the derived binding energy of NO to FeTPyrPH$^+$ and to FeTPyrPH$_2^+$ estimated from the observed radiative association rates using Dunbar's "standard hydrocarbon" method of analysis[3] are listed in Table 1. Numbers from this method of analysis are found to be typically accurate to ±0.2 eV (±4.6 kcal/mol).[3]

Increasing the temperature decreases the association rate, and in the case of FeTPyrPH$_2^+$ at an NO pressure of 2×10^{-7} Torr the result is that competition between association and dissociation brings the free metalloporphyrin and its complex into equilibrium as shown in Figure 1. While at low-temperature FeTPyrPH$_2^+$ disappears completely in favor of FeTPyrPH$_2$(NO)$^+$, at higher temperatures the FeTPyrPH$_2^+$ concentration approaches a constant nonzero value at long time as the association comes to equilibrium. The equilibrium constant can be obtained from the NO pressure and the ratio of the steady-state signal intensities of FeTPyrPH$_2$(NO)$^+$ and FeTPyrPH$_2^+$. The ratio of the FeTPyrPH$_2$(NO)$^+$ and FeTPyrPH$_2^+$ signals at equilibrium are obtained by fitting the curves to functions consisting of the sum of an exponential and a constant. The curves in the figures are labeled with average temperatures obtained from two thermocouples mounted to the cell. Because of the way the vacuum system is heated (to protect a signal preamplifier) there is a temperature gradient across the cell, giving an uncertainty in assigning the temperatures ranging from about ±5K to ±10K. Furthermore the range of temperatures over which the equilibrium constant can be measured is very small, making a Van't Hoff analysis inappropriate. Nevertheless it is clear from the data in Figure 1 that the system comes to equilibrium at the higher temperatures. The free energy of binding determined from the equilibrium constants and the average temperatures combined with an entropy estimated from statistical mechanics[11] gives the binding energy. The equilibrium constant at one nominal temperature and the derived binding energy are listed in Table 1. The binding energies obtained from the equilibrium constants at the various nominal temperatures agree to within ±0.3 kcal/mol. The estimated error derives from the uncertainty in temperature and the uncertainty in the estimated entropy. We note that the equilibrium constant listed in Table 1 corresponds to a $P_{1/2}$(NO), the NO pressure at which FeTPyrPH$_2^{+2}$ and FeTPyrPH$_2$(NO)$^+$ are equally abundant, of 3.8×10^{-7} Torr. If the solvation energies required to move

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Table 1. Measured Parameters and Derived NO Binding Energies

species (M)	k_a 10^{-11} cm^3 s^{-1}	K_{eq} 10^9 atm^{-1}	E_A kcal/mol	D (M−NO) kcal/mol	method
$FeTpyrPH_2$+2	4.8 ± 1.5 (288K)	2.0 ± 0.6 (336K)		26.6 ± 0.7[a]	radiative association[b]
				28.9 ± 1.5[c]	associative equilibrium[b]
$FeTpyrPH^+$	4.8 ± 1.5 (288K)			26.6 ± 0.7[a]	radiative association[b]
$FeTPP^+$			10.0 ± 0.9	25.9 ± 0.9[d]	blackbody radiative dissociation[b]
$heme^+$	1.5 ± 0.5 (288K)			24.8 ± 0.7[a]	radiative association[b]
FePorphyrin				35	DFT[e]

[a] From uncertainties of rate constants and temperature (±5K). The analysis method (4) is considered to be accurate to ±0.2 eV or ±4.6 kcal/mol. [b] Present results. [c] Uncertainty from uncertainties in the temperature (±7K) and the entropy estimate. [d] Uncertainty from Arrhenius plot. [e] Result from ref 7.

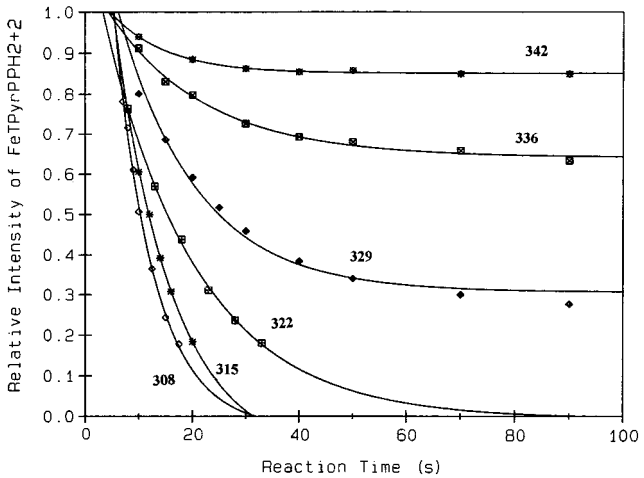

Figure 1. Disappearance with time of $FeTPyrPH_2^{+2}$ as a result of addition of NO at various temperatures. Lines are exponential fits to the data. RF pulses to isolate reactant ions leaves them translationally excited. Hence, there is an induction period during which the ions are cooled before exponential decay begins.

$FeTPyrPH_2^{+2}$ and $FeTPyrPH_2(NO)^+$ from the gas phase into solution are similar, $P_{1/2}$(NO) should be similar for the association equilibrium in solution. Henry's Law indicates that an NO pressure of 3.8×10^{-7} Torr corresponds to an equilibrium concentration of NO in aqueous solution of approximately 1×10^{-12} M. Our results thus suggest that NO concentrations in the sub-picomolar range could therefore be physiologically significant.

$P_{1/2}(O_2)$, the partial pressure of O_2 above the solution corresponding to equal equilibrium concentrations of the free and O_2 bound metal complex, is generally reported in studies of the binding of O_2 to metalloporphyrins and heme proteins in solution.[12] Measurements of values of $P_{1/2}$ smaller than 10^{-4} Torr are not practical using the usual methods.[13] This, in part, is the reason that there is no $P_{1/2}$(NO) for the association of NO with metalloporphyrins and heme proteins in solution to be compared with our gas-phase result.

The ions produced by electrospray of myoglobin were also allowed to react with NO. The electrospray spectrum of myoglobin contained myoglobin with predominantly 8−10 protons, stronger signals of apomyoglobin with predominantly 5−12 protons, and free heme with +1 charge but no protons. Of these species only the free heme was observed to add NO at reaction times out to more than 30 s at 2×10^{-7} Torr of NO. The bond energy between NO and the positively charged heme deduced from the radiative association rate is given in Table 1. The failure of NO to add to myoglobin suggests that the bond between NO and myoglobin may be weaker than the bond between NO and the free positively charged heme. This could be the result of the interaction of the heme with nearby histidine residues in myoglobin. It may also be that the NO does not sample efficiently the binding site in gas-phase collisions with the large and complex myoglobin ion.

The iron tetraphenylporphyrin cation, $FeTPP^+$, was produced by electrospray ionization of iron tetraphenylporphyrin chloride and found not to add to NO. The NO complex of iron tetraphenylporphyrin cation ($FeTPP^+$) was produced, however, in a Nicolet FTMS-2000 FT-ICR-MS by the two-step reaction of that ion with NO_2 ($FeTPP^+ + NO_2 \rightarrow FeTPPO^+ + NO$, $FeTPPO^+ + NO_2 \rightarrow FeTPPNO^+ + O_2$).[6] The complex was then transferred to a differentially pumped adjacent cell[14] at low pressure ($\sim 10^{-8}$ Torr) where it was observed to dissociate to $FeTPP^+$ and NO with a unimolecular rate constant dependent on temperature. At low pressure, the exchange of infrared radiation with the walls of the chamber is the primary means by which activation of a reactive molecule can occur.[4,5] Under these circumstances, the meaning of the activation energy (E_a) depends on the size of the system and how fast it reacts. The $FeTPPNO^+$ system falls into the "small molecule" category[4c] where the rate of ion activation by the radiation field is slow relative to the rate of reaction. This distorts the internal energy distribution of the reacting molecules, and a correction must be made to the E_a to obtain the binding energy. The procedure for making this correction has been outlined by Dunbar[4a,b] and further described by McMahon and Dunbar.[4c] The bond energy between $FeTPP^+$ and NO found using the McMahon and Dunbar procedure is listed in Table 1.

Taking the equilibrium measurement as the most reliable since it is independent of kinetic models suggests that the bond energies deduced from the radiative association rates may be somewhat too small, but the agreement is satisfactory considering the approximate nature of the analysis of the radiative association rate data. The radiative association numbers suggest that charge on the ligand plays little role in the interaction since the singly and doubly protonated FeTPyrP species give essentially the same result. The radiative association numbers also suggest that the heme ion binds NO just about as strongly as do the FeTPyrP ions, suggesting further that the bond strength is not strongly dependent on details of ligand structure. The agreement between the bond strength to $FeTPP^+$ determined by the BIRD experiment and the numbers from radiative association tends to validate both methods and to suggest that $FeTPP^+$ and the protonated FeTPyrP ions interact similarly with NO. The bond strength between NO and iron porphyrin has recently been calculated using density functional theory (DFT) methods to be 35 kcal/mol.[7] This agrees reasonably well with the present results.

The metalloporphyrin systems examined failed to form adducts when exposed to O_2 and CO. This is consistent with what is known about the binding energies of O_2 and CO to metalloporphyrins. Rovira, et al. found in their DFT calculations binding energies of 9 and 26 kcal/mol FeP−O_2 and FeP−CO, respectively, whereas they found a binding energy of 35 kcal/mol for FeP−NO, where FeP is iron porhyrin and the ligands are bound to the metal.

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(11) Loss of translational (−41.2 cal K^{-1} mol^{-1}), rotational (−9.0 cal K^{-1} mol^{-1}) and electronic entropy ($-R$ ln(3) = −2.2 cal K^{-1} mol^{-1}, see ref 7) plus the estimated entropy of low-frequency vibrations of bound NO (7.1 cal K^{-1} mol^{-1}, based on frequencies in Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B: Applications in Coordination, Organometallic, and Bioinorganic Chemistry*, 5th ed.; Wiley: New York, 1980) and gives $\Delta S = -45.3$ cal K^{-1} mol^{-1}. Uncertainty assigned = ± 4.0 cal K^{-1} mol^{-1}.

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