Equilibrium and Kinetic Study of Nitric Oxide Binding to Phthalocyaninatoiron(II) in Dimethyl Sulphoxide

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The equilibrium and kinetics of the reaction between nitric oxide and phthalocyaninatoiron(II), [Fe(pc)], in dimethyl sulphoxide (dmso) has been studied at 20 ± 0.5 °C. In the presence of a large excess of NO, [Fe(pc)] binds nitric oxide in a 1 : 1 mol ratio *via* pseudo-first-order kinetics. The observed rate constant has the general form $k_{obs.} = k_f[NO]$, *i.e.* with an intercept close to zero and a slope $k_f = (2.2 \pm 0.4) \times 10^4$ dm^3 mol^{-1} s^{-1}. From the values of the equilibrium constant [$K = (1.1 \pm 0.1) \times 10^6$ dm^3 mol^{-1}] and second-order rate constant (k_f) for the binding of NO, the dissociation rate constant (k_r) has been estimated to be $(2.0 \pm 0.4) \times 10^{-2}$ s^{-1}. The present results are discussed in the light of related previous investigations.

Phthalocyaninatoiron(II), [Fe(pc)], widely investigated as a porphyrin-like molecule, binds a number of small molecules such as O_2, CO, or NO under a variety of conditions.[1a–g] A kinetic study of the reaction of [Fe(pc)] with O_2 in dimethyl sulphoxide (dmso) showed the mechanism to be complex;[1c] in contrast, a simple and reversible binding of CO to [Fe(pc)], in the presence and absence of pyridine, has been reported in the same solvent.[1d,e] On the other hand, no detailed investigation has been reported so far for the interaction of [Fe(pc)] with NO in a homogeneous medium, if exception is made for the qualitative description previously given in 96% H_2SO_4.[1g]

This paper presents the results of a study on the equilibrium and kinetics of the reaction between [Fe(pc)] and nitric oxide in dmso. The results are discussed in the light of what is known in the literature on the reaction of nitric oxide with porphyrin systems and haemoproteins.[2,3]

Experimental

Materials.—Phthalocyaninatoiron(II) was purchased from Eastman Kodak Co. (Rochester, New York, U.S.A.), and purified as previously described.[1d] Solid [Fe(pc)(NO)] was easily obtained by suspending [Fe(pc)] in tetrahydrofuran in an atmosphere of NO under stirring at room temperature for 24 h. Dimethyl sulphoxide (Merck A.G., Darmstadt, W. Germany; spectrograde) was distilled under reduced pressure over CaH_2 before use and stored in a desiccator. Nitric oxide was obtained from B.D.H. Chemicals Ltd. and carbon monoxide purchased from SIO S.p.A. (Rome, Italy).

Solubility of Nitric Oxide in Dimethyl Sulphoxide.—As far as we know, no literature data are available for the solubility of NO in dmso. Thus, the NO concentration in this solvent was determined at 20 ± 0.5 °C and a pressure of 760 Torr (*ca.* 10^5 Pa), by titration with iron(II) horse myoglobin (mb) which is known readily to bind NO in a 1:1 stoicheiometry.[2] The increase in absorbance at 420 nm as a function of the added volume of dmso (kept in a closed vessel under NO at 760 Torr) is reported in Figure 1. Reproducibility of the results was better than ±3%. The solubility of NO in dmso at 20 ± 0.5 °C was found to be 3.0×10^{-3} mol dm^{-3}. This value is of the expected order of magnitude when compared with solubility data obtained in other solvents.[4]

Equilibrium Measurements.—A solution of [Fe(pc)] (1.35×10^{-7} mol dm^{-3}), prepared by dissolving the complex in dmso under an inert atmosphere (N_2), was introduced anaerobically into a 1-cm spectrophotometric cell fitted with a serum cap for the injection of the NO solution (no gaseous phase was present). Various aliquots of dmso (kept in a closed vessel under NO at 760 Torr) were added to the [Fe(pc)] solution by using a precision microsyringe. At the [Fe(pc)] concentration used in the experiments, the fraction of NO bound to the iron complex was always negligible compared to the total ligand concentration. After each addition, the system was equilibrated at 20 ± 0.5 °C, this requiring less than 5 min, and the spectrum recorded on a Varian Cary 219 spectrophotometer. Figure 2(*a*) shows the spectral changes observed in a typical experiment.

The reaction is completely reversible, since the spectrum reverts to the initial one by merely pumping off the nitric oxide or bubbling nitrogen through the solution.

Kinetic Measurements.—Measurements of NO recombination rate after photolysis were carried out using a cross-illumination apparatus equipped with a steady-state light source and a flash lamp, as described by Brunori and Giacometti.[5] The photolysing light was the total output of a 300-J flash lamp. Variation of the intensity of the photolysing light was achieved by interposing calibrated neutral density filters (transmission from 5 to 50%).

Measurements were carried out at 20 ± 0.5 °C using [Fe(pc)] solutions (6.0×10^{-6} mol dm^{-3}) equilibrated with NO at known pressures. The rate of the recombination reaction after photolysis was measured at different wavelengths in the

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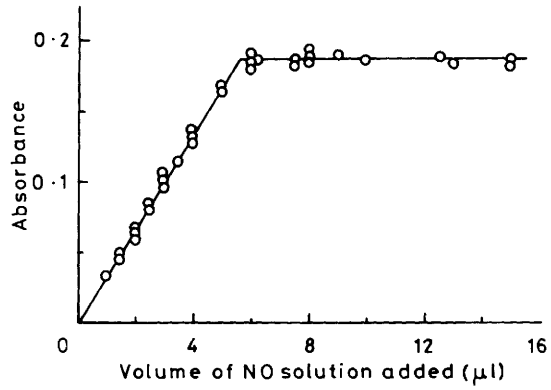

Figure 1. Titration of deoxygenated horse myoglobin with nitric oxide in dmso at 20 ± 0.5 °C. The increase in absorbance at 420 nm of a 4.79×10^{-6} mol dm^{-3} solution of horse mb (in 0.1 mol dm^{-3} phosphate buffer at pH 7.0) is plotted against the volume of the NO solution in dmso added to a fixed volume (3 498 μl) of the mb solution. Dimethyl sulphoxide was previously equilibrated with pure NO at 760 Torr and 20 °C. From the break point (equivalence point) and the known stoicheiometry for the binding of NO to iron(II) horse mb (*i.e.*, 1:1),[2] the concentration of the gas in dmso (3.0×10^{-3} mol dm^{-3}) can be calculated

range 630—690 nm. Photolysis by monitoring light was negligible and thus corrections were not necessary.

Results and Discussion

Equilibrium Measurements.—[Fe(pc)] gives a mononitrosyl derivative, *i.e.* [Fe(pc)(NO)], by reaction with nitric oxide,[1f] similarly to porphyrinatoiron(II) complexes.[2,3] The reaction between [Fe(pc)] and NO in dmso may be written as equation (1), where axial and free solvent molecules are omitted for

$$[\text{Fe(pc)}] + \text{NO} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} [\text{Fe(pc)(NO)}];\ K = k_f/k_r \quad (1)$$

simplicity. The spectral changes observed when a 1.35×10^{-7} mol dm^{-3} [Fe(pc)] solution was titrated with NO [see Figure 2(*a*)] were analysed in order to obtain the equilibrium constant. Figure 2(*b*) shows the plot of log [α/(1 − α)] against log[NO] at 20 ± 0.5 °C, where α is the fraction of nitrosylated [Fe(pc)]. Analysis of the data[2] yields log$K$ = 6.04 ± 0.04 and slope n = 1.07 ± 0.07 (uncertainties are standard deviations), the latter being in full agreement with the stoicheiometry of equation (1). Consistently, the final spectrum observed was found to be identical to that obtained by dissolving [Fe(pc)(NO)] in dmso.

As reported for the binding of gaseous ligands to haemoproteins and/or haem model compounds,[2,3] the equilibrium constant for NO association to [Fe(pc)] in dmso [(1.1 ± 0.1) × 10^6 dm^3 mol^{-1}] is higher than that observed for the formation of the [Fe(pc)(CO)] adduct in the same solvent, both in the presence [(6.5 ± 0.5) × 10^3 dm^3 mol^{-1}] and absence [(1.4 ± 0.3) × 10^4 dm^3 mol^{-1}] of pyridine.[1d,e]

Kinetic Measurements.—The intensity of the 300-J flash lamp (see above) induced partial photodissociation of [Fe(pc)(NO)], as shown by the fact that the amplitude of the kinetic difference spectrum of [Fe(pc)(NO)] *minus* [Fe(pc)] is less than that obtained in static measurements [see Figure 3(*a*)]. Nevertheless, the profiles of the static and kinetic difference spectra of [Fe(pc)(NO)] *minus* [Fe(pc)] match each other [see Figure 3(*a*)], thus indicating the absence of significant amounts of spectroscopic intermediates.

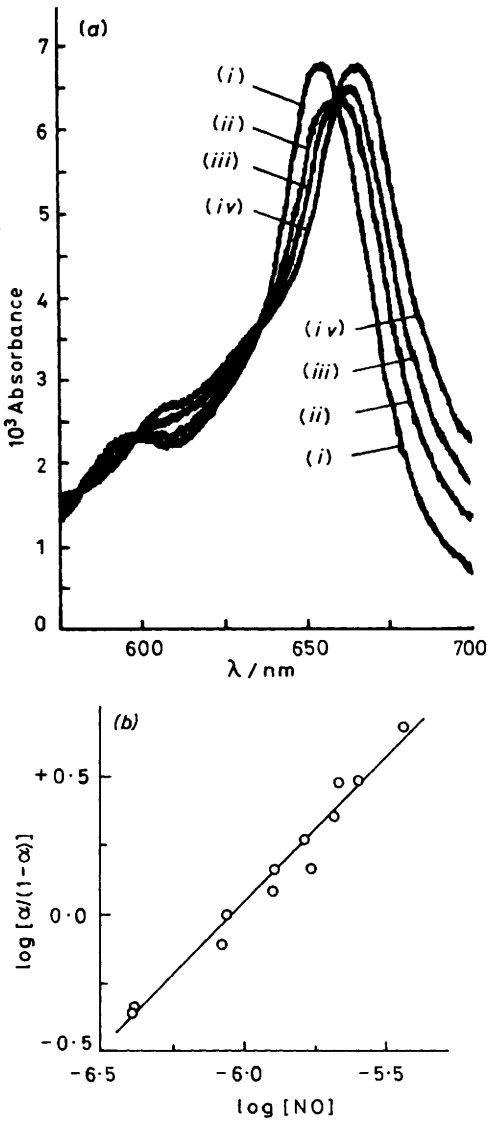

Figure 2. (*a*) Spectral changes due to the binding of NO to a 1.35×10^{-7} mol dm^{-3} [Fe(pc)] solution in dmso at 20 ± 0.5 °C: [NO] = 0 (*i*), 8.6×10^{-7} (*ii*), 1.7×10^{-6} (*iii*), or 8.6×10^{-5} mol dm^{-3} (*iv*). (*b*) Plot of log[α/(1 − α)] against log[NO] at 20 ± 0.5 °C. The line was calculated according to the equation[2] log[α/(1 − α)] = − log$K$ + $n$log[NO], with log$K$ = 6.04 and n = 1.07

The dependence on light intensity of the photodissociation of nitric oxide and carbon monoxide derivatives of [Fe(pc)] in dmso has been analysed in parallel [see Figure 3(*b*)], in order to determine the relative quantum yield. If recombination of the photolysed partners is negligible during the flash (*i.e.*, no geminate recombination occurs), the decrease in concentration of the [Fe(pc)(X)] (X = NO or CO) complex at the end of the pulse of light can be expressed according to equation (2),[5] where

$$\ln\{[\text{Fe(pc)(X)}]_0/[\text{Fe(pc)(X)}]_i\} = \omega\gamma i \quad (2)$$

[Fe(pc)(X)]$_0$ and [Fe(pc)(X)]$_i$ represent the concentration of the adduct before and after the pulse of light, respectively, ω is a proportionality constant representing the overall light absorbance by the sample over the emission spectrum characteristic of the flash (white light), γ is the quantum yield, and i is the light

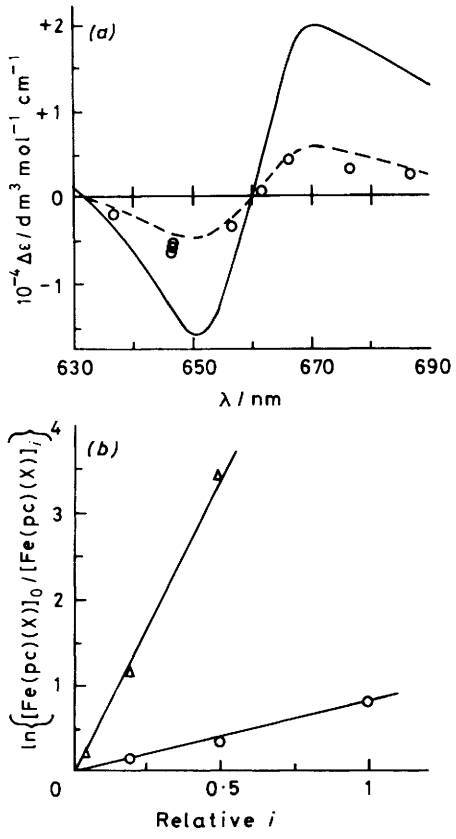

Figure 3. (*a*) Static difference spectrum of [Fe(pc)(NO)] *minus* [Fe(pc)] (continuous line) in dmso at 20 ± 0.5 °C. Circles represent the amplitude of the optical density changes observed in the kinetic experiments. The dashed line is the best static difference spectrum of [Fe(pc)(NO)] *minus* [Fe(pc)] fitting optical density changes observed in the kinetic experiments. (*b*) Dependence of the photodissociation of the NO (○) and the CO (△) derivatives of [Fe(pc)] in dmso on the light intensity at 20 ± 0.5 °C. Straight lines for NO and CO photodissociations were calculated according to equation (2) with $\omega\gamma$ = 0.8 and 6.7 for the NO and CO derivatives, respectively

intensity. The product $\omega\gamma$ may be obtained from the slope of plots of $\ln\{[Fe(pc)(X)]_0/[Fe(pc)(X)]_i\}$ *versus* *i*, as shown in Figure 3(*b*). Since the absorption spectra of [Fe(pc)(NO)] and [Fe(pc)(CO)] [1d] are fairly similar [*i.e.*, ω(CO) ≃ ω(NO)], the quantum yield for the photodissociation of NO relative to that of the CO derivative may be obtained from the slopes in Figure 3(*b*). If one assumes that the absolute quantum yield for the photodissociation of the [Fe(pc)(CO)] complex is between 0.3 and 1, *i.e.* similar to that reported for carbonylated haemoproteins and haem model compounds,[3,5] then the photodissociation of the [Fe(pc)(NO)] adduct may be estimated to have a value of γ in the range 0.036—0.12. These values may be somewhat higher than those observed for the photodissociation of NO haemoproteins ($\simeq 1 \times 10^{-3}$),[5] but compare fairly well with values reported for the NO derivatives of other haem model compounds (γ = 0.05—0.1).[3]

In flash photolysis experiments, in the presence of a large excess of nitric oxide, the time course of NO recombination was always found to be first order, the rate constant being linearly dependent on [NO] with a negligible intercept. The values of the second-order rate constants calculated at different NO concentrations are reported in the Table; a value of k_f = (2.2 ± 0.4) × 10^4 dm^3 mol^{-1} s^{-1} can be determined as an average (uncertainty is half dispersion).

Table. Second-order rate constants for the recombination reaction, after flash photolysis, of nitric oxide with [Fe(pc)] in dmso (θ_c = 20 ± 0.5 °C)

[NO]/mol dm^{-3}	$10^{-4}k_f$*/dm^3 mol^{-1} s^{-1}
3.0 × 10^{-3}	2.1 ± 0.3
2.6 × 10^{-4}	1.9 ± 0.2
2.3 × 10^{-5}	2.7 ± 0.3

* Average values from independent experiments; uncertainties are half dispersions.

The value of the NO dissociation rate constant from the [Fe(pc)(NO)] adduct has been estimated from the equilibrium constant (*K*) and the second-order rate constant (k_f) for nitrosylation of [Fe(pc)] according to reaction (1); thus a value of k_r = (2.0 ± 0.4) × 10^{-2} s^{-1} is obtained.

The overall second-order dependence of the rate of nitrosylation of [Fe(pc)] is not inconsistent with a dissociative mechanism, as discussed elsewhere.[1d,e,6]

Somewhat similarly to that reported for haemoproteins and haem model compounds,[2,3] the value of k_f for nitric oxide binding to [Fe(pc)] is higher than that of carbonylation of the same macrocycle, in the presence [(8.8 ± 0.5) × 10^2 dm^3 mol^{-1} s^{-1}] and absence [(1.28 ± 0.05) × 10^3 dm^3 mol^{-1} s^{-1}] of pyridine;[1d,e] similarly, the value of k_r is lower than that for [Fe(pc)(CO)] dissociation, in the presence (0.16 ± 0.03 s^{-1}) and absence (0.12 ± 0.02 s^{-1}) of pyridine.[1d,e]

As a whole, the data here presented for NO binding to [Fe(pc)] in dmso together with data already reported for the corresponding carbonylation [1d,e] indicate that NO and CO bind with a simple mechanism, reminiscent of that reported for nitrosylation, carbonylation, and oxygenation of monomeric haemoproteins and haem model compounds.[2,3,5] On the other hand, oxygen binding to [Fe(pc)] in dmso proceeds through a more complex mechanism ultimately leading to the formation of a μ-oxo adduct.[1c] However, this seems to be related to the tendency of the dioxygen adduct to bind another [Fe(pc)] molecule and to the instability of the so formed μ-peroxo dimer rather than to the initial binding of the diatomic ligand. More extended investigations on the role of the structural parameters affecting the reactivity of [Fe(pc)] (such as axial ligands and solvent as well as peripheral macrocycle substituents) may be of interest.

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