Transient Oxygenation of Chelated Iron(II) Porphyrins: Improved Kinetics of Carbon Monoxide Replacement by Oxygen

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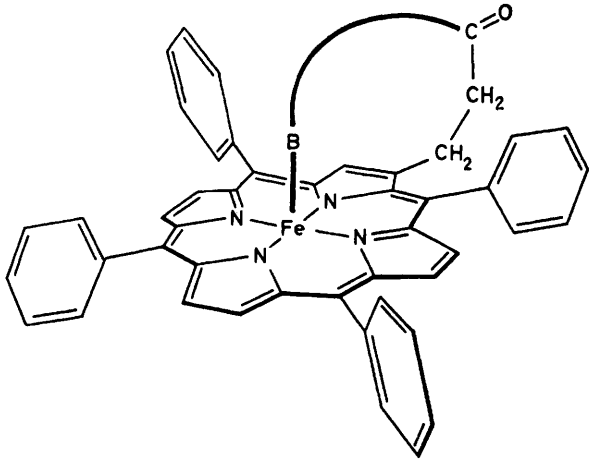
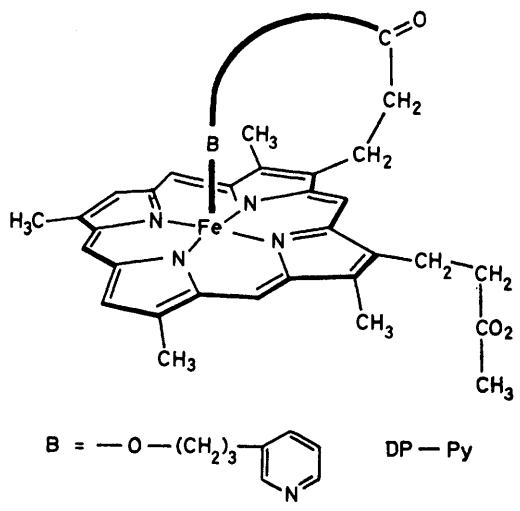
The kinetics of CO replacement by O_2 (Gibson's equation) cannot be applied without modification to the study of unstable oxyheme model compounds which bind oxygen weakly. A generalization of Gibson's equation is proposed. Using the time resolution offered by laser photolysis techniques, the oxygen ' off ' rates are determined both from the fast oxygen-binding phase following photodissociation of the carboxyhemochrome as well as from the modified kinetics of the subsequent ligand exchange. Applications to chelated tetraphenylporphyrin and deuteroporphyrin model compounds are reported.

THE determination of the rate constants for the reaction of oxygen with iron(II) porphyrins is directly relevant to the elucidation of the mechanisms of oxygen uptake and release by naturally occurring heme proteins. The design of so-called ' model oxygen carriers ' must however overcome two major difficulties. First, the porphyrin has to be prepared as a stable pentaco-ordinate deoxyheme. This can be achieved by attaching covalently an axial ligand to the porphyrin moiety in such a way as to permit a strain-free intramolecular co-ordination with the iron atom.[1-4] Such pentaco-ordinated deoxyhemes, carrying imidazole or pyridine as a first axial ligand, are able to combine with oxygen to give hexaco-ordinated species for which the name ' oxyhemochrome ' has been proposed.[5] A second difficulty arises from the irreversible auto-oxidation of oxyhemochromes into iron(III) μ-oxo-dimers.[6] Except in very polar solvents, this undesirable reaction is almost immediate at room temperature and thus makes a direct study of oxyhemochromes impracticable.

An indirect, but elegant, solution to the problem is offered by the technique of photo-triggered ligand replacement which was originally introduced for investigating heme proteins.[7,8] In contrast to oxyhemochromes, hexaco-ordinated complexes containing carbon monoxide (carboxyhemochromes) are extremely stable, even in the presence of a large amount of dissolved oxygen. Another characteristic is their easy photodissociation into carbon monoxide and pentaco-ordinated porphyrin. Whereas pentaco-ordinated iron(II) porphyrins react faster with oxygen than with carbon monoxide, their overall affinity for the latter ligand is larger. Consequently the oxyhemochrome which is formed at first undergoes later on a ligand replacement reaction which restores the initial carboxyhemochrome. Although the ligand exchange is a relatively slow process, it is faster than auto-oxidation. As a consequence many experiments can be successively performed without any significant porphyrin degradation.

A well known equation (sometimes known as Gibson's equation) relates the relaxation time for exchange to the ' on ' rates of CO and O_2 and to the oxygen ' off ' rate constant.[8] The kinetic derivation of the original work uses some approximations which are equivalent to assuming a large equilibrium constant for oxygen binding. This was no doubt correct for heme proteins, but the assumption has remained unquestioned in





subsequent works dealing with heme model compounds.[1,2]

We report in the present paper on the reactivity of oxygen towards simple model compounds derived from 5,10,15,20-tetraphenylporphyriniron(II) and (deuteroporphyrin IX)iron(II).

These investigations soon convinced us that the determination of the oxygen ' off ' rates can be grossly in error if the equilibrium constant is small ($\leqslant 10^3$ l mol^{-1}). In view of the frequent use of the ligand replacement kinetics in the literature, we suggest a generalization of Gibson's equation which takes this weak binding case into account.

EXPERIMENTAL

5,10,15,20-Tetraphenyl-1-[3-(*N*-imidazoylpropyl)propionamido]porphyriniron(III) (TPP-Im) and 5,10,15,20-tetraphenyl-1-[2-(3-pyridyl-n-propoxy)carbonylethyl]porphyriniron(III) (TPP-Py) were synthesized and purified as previously described.[4] 7(6)-[2-(3-Pyridyl-n-propoxy)]-deuteroporphyriniron(III) 6(7)-methyl ester (DP-Py) was prepared first by coupling 3-(3-pyridyl)propanol with deuterohemin IX through one of the propionic side chains in the presence of dicyclohexylcarbodi-imide and then following the procedure published elsewhere.[3]

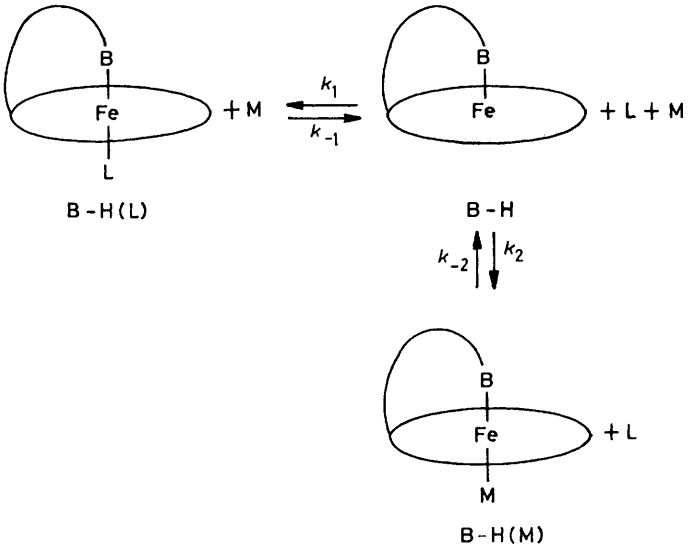
The hemins (5×10^{-5}M in toluene) were reduced by aqueous sodium dithionite under argon. The reduced solution was transferred anaerobically into the optical cuvette through a latex septum and bubbled for 30 min with pure carbon monoxide. This procedure removed traces of water [9] and the carboxyhemochrome formed was flashed in order to measure k_1, the rate constant for CO binding. Several CO concentrations were used by bubbling successively with different argon–CO mixtures. The measurements of the exchange rate were performed after bubbling the solution with CO–O_2 mixtures using calibrated Brook's flow-meters. The concentration range was 1—4mM for O_2 and 0.6—0.2mM for CO [gas solubilities in toluene at 20 °C were taken as 5.32mM atm^{-1} (O_2) and 7.2mM atm^{-1} (CO) respectively].

Our laser flash photolysis apparatus has been described in detail elsewhere.[10] The carboxyhemochromes were photodissociated using the second harmonic (532 nm) of a pulsed neodymium laser (pulse width 20 ns). The absorbance changes were monitored at the isosbestic wavelength between carboxyhemochrome and photodissociated pentaco-ordinated porphyrin (in the region 420—460 nm). The position of the isosbestic point was determined by repeatedly flashing the sample until the wavelength was found at which the initial absorbance change was equal to zero. The formation of oxyhemochrome was characterized by a rapid absorbance increase in the first 0.5—5 μs and was followed later on by the slow ligand exchange during which the signal returned to the base line (ms or more).

RESULTS AND DISCUSSION

Let two different ligands L and M bind to a penta-co-ordinated heme B–H in which –H represents the reduced hemeiron(II) and B an internally chelated base. The equilibria in the Scheme compete.

The rate constants k_1 and k_2 are pseudo-first-order rate constants $k_1 = k'_1[L]$ and $k_2 = k'_2[M]$, provided the concentration of ligand is much larger than the heme concentration. For all systems of interest we may assume that, even in the presence of an excess of M, the total heme appears as hexa-co-ordinated B–H (L). Then k_{-1} can be neglected. The approximation is largely satisfied for L = CO since the off-rate of carbon monoxide is of the order of min^{-1}.



SCHEME

Photodissociation of L leads to the penta-co-ordinated B–H species and the reappearance of B–H (L) is governed by the rate equations associated with the Scheme. Usually, the recombination kinetics show two distinct exponential phases. During the first, fast phase L and M compete for binding and a certain amount of B–H (M) is present at the end of this step. This phase is characterized by a relaxation time τ_2 for the formation of B–H (M) and can be followed accurately at the isosbestic wavelength for B–H (L) and B–H. The second, slower, phase with relaxation time θ, corresponds to the ligand exchange leading back to B–H (L) *via* the penta-co-ordinated B–H according to the Scheme.

Both the relaxation times are calculated by solving the differential rate equations associated with the Scheme. They are found to be the roots of equation (1) and well

$$x^2 + (k_1 + k_2 + k_{-2})x + k_1k_{-2} = 0 \qquad (1)$$

separated exponential phases can be observed when $k_1k_{-2} \ll k_1 + k_2 + k_{-2}$. An important relation can be derived from equation (1), namely that the product of the relaxation times must remain equal to $(k_1k_{-2})^{-1}$. Hence equation (2) applies where we have introduced the

$$\theta = \frac{1}{k_{-2}} \cdot \frac{\tau_1}{\tau_2} \qquad (2)$$

relaxation time τ_1 for the recombination of L in the absence of M: $(\tau_1)^{-1} = k_1 = k_1'[L]$.

Relation (2) is the basis for a purely kinetic determination of k_{-2}. It is valid under all circumstances and

involves only the measurement of the relaxation time τ_1 in the absence of M and of the two relaxation time θ and τ_2 with both L and M present. This can be achieved within a few minutes provided the detection system allows measurements in widely differing time ranges, since typically τ_2 *ca.* 10^{-7}—10^{-5} s, τ_1 *ca.* 10^{-5}—10^{-3} s, and θ *ca.* 10^{-3}—10^{-1} s. An advantage is that no extrapolation procedure is required.

With the approximation mentioned, the general solution of the Scheme yields equation (3). The ' on ' rate k'_2 is obtained from the slope of a plot of relation (3). The presence of an appreciable intercept is characteristic of the weak binding case (large value of k_{-2}) and provides one with a second determination of k_{-2}.

$$(\tau_2)^{-1} - (\tau_1)^{-1} = k_{-2} + k_2'[\mathrm{M}] \qquad (3)$$

room temperature. On the average, the oxygen molecule remains only *ca.* 10 μs on a given heme. The equilibrium constants $K_2 = k'_2/k_{-2}$ are in the range 1—3 × 10^{-3} l mol^{-1}.

Two closely related models, pyroheme–Py and mesoheme–Im, have been previously investigated by Traylor and his co-workers,[1,2,11] using conventional flash photolysis. Their results have been included in the Table for the sake of comparison. Surprisingly their ' off ' rates are one or two orders of magnitude smaller than those reported here. We thought at first that the difference could be due to the porphyrin *cis*-effect owing to the presence of the phenyl substituents in TPP-hemes, but our deuteroheme model shows the same discrepancy. Another possible explanation could be a conformational relaxation of the newly formed oxygen complex tending

CO and O_2 binding rate parameters for chelated heme models in toluene (20 °C)

	CO	O_2			
			$10^{-4}\ k_{-2}/s^{-1}$		
	$10^{-6}\ k'_1$/l mol^{-1} s^{-1}	$10^{-7}\ k'_2$/l mol^{-1} s^{-1}	*a*	*b*	$10^{-3}\ K_2$/l mol^{-1}
TPP-Im	4.4	5	3	2.5	1.7
TPP-Py	6.5	10	9	11	1.1
DP-Py	12	20	8	7	2.5
Meso-Im [c]	8	5.3		0.17	30
Pyro-Py [d]	7.5	0.35		0.25	1.4

[a] From the initial oxygen binding kinetics using equation (3). [b] From exchange with CO [equation (2)]. [c] From ref. 2. [d] From ref. 1.

To show the connection with Gibson's equation, equation (2) can be rewritten as (4). When k_{-2} is small

$$\theta - \tau_1 = \frac{1}{k_{-2}}\left(1 + \frac{k_2'[\mathrm{M}]}{k_1'[\mathrm{L}]}\right) \qquad (4)$$

(strong binding) then the exchange rate is very slow. If τ_1 is neglected in the left side, equation (4) becomes identical with Gibson's equation. Usually the relaxation time for exchange is plotted against the ratio [M]/[L] and k_{-2} is the intercept of the plot. When k_{-2} is large (weak binding), θ and τ_1 may happen to be of the same order of magnitude. The use of the graphical procedure based on the complete relation (4) then becomes rather inaccurate. We therefore preferred to treat the data according to the general relations (2) and (3) which require no *a priori* assumption.

In agreement with equation (3), the plots of the fast relaxation time for oxygen binding (τ_2) against oxygen concentration were perfectly linear for all three heme models investigated. A non-zero intercept was also distinctly observed showing that weak binding occurred. From these plots we obtained k'_2 and a first estimate of k_{-2}. The exchange relaxation time θ measured at the same O_2 and CO concentrations allowed us to compute another set of k_{-2} values according to the general relation (2). Both determinations were in close agreement as shown by the results listed in the Table.

The dissociation rate is a most appropriate measure of the intrinsic stability since it is directly related to the lifetime of a complex. It turns out that the model heme compounds bind oxygen rather poorly in toluene at towards a greater stability, and hence lower k_{-2}, over a period of the order of milliseconds. Indeed k_{-2} as obtained from the fast oxygen binding curve [equation (3)] refers to the complex during the first few microseconds after binding O_2 to the penta-co-ordinated B–H. In the work [1,2] on meso- and pyro-heme models, k_{-2} could be obtained only from the plot of the slow exchange relaxation time using Gibson's equation. Due to the long duration of the flash in these experiments the ligand concentrations had to be kept low in order to slow down the whole reaction. The ' off ' rates determined in this way therefore referred to oxygenated heme that had been formed several milliseconds before.

However the agreement found in the present work between the determination of k_{-2} both at short and at longer times seems to eliminate such an (unlikely) relaxation mechanism. We rather believe that the lower ' off ' rates reported in refs. 1 and 2 presumably result from the failure of the strong-binding kinetics to describe the behaviour of these heme models in non-polar organic solvents. Further work using similar models embedded in aqueous micelles [11] seems to indicate that the combination rates are little affected, but that the overall oxygen affinity is increased in such systems because of a much lower dissociation rate. This supports the view that external factors such as solvent polarity play a significant role in stabilizing the iron–oxygen bond.

New models are under preparation in order to investigate whether intramolecular polar groups could be as effective for stabilizing the oxyhemochromes.

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