Pulse Radiolytic Studies of Metallotexaphyrins in the Presence of Oxygen: Relevance of the Equilibrium with Superoxide Anion to the Mechanism of Action of the Radiation Sensitizer Motexafin Gadolinium (Gd−Tex^{2+}, Xcytrin)

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Pulse radiolytic studies of aqueous solutions of four representative metallotexaphyrin complexes M−Tex^{2+} (M = Gd(III), Lu(III), Dy(III), and Y(III)), carried out in the presence of either dioxygen, or trimethyl-*p*-benzoquinone (TMQ). It was found that all four of these M−Tex^{2+} species set up an equilibrium with superoxide and the singly reduced TMQ (TMQ$^{\bullet-}$) on the pulse radiolytic time scale. Rate constants for the forward (k_1) and back (k_{-1}) reactions of Gd−Tex^{2+} with superoxide anions, at pH 8.5, were determined to be $(9.8 \pm 1.5) \times 10^6$ M^{-1} s^{-1} and $(3.4 \pm 0.5) \times 10^6$ M^{-1} s^{-1}, respectively. For reaction with TMQ$^{\bullet-}$, the analogous rate constants were found to be $(1.5 \pm 0.2) \times 10^7$ M^{-1} s^{-1} and $(3.7 \pm 0.6) \times 10^6$ M^{-1} s^{-1}, respectively. Equilibrium constants (K_{kin}), calculated from these kinetic parameters, were found to be 2.9 and 4.1 for Gd−Tex^{2+} reacting with $O_2^{\bullet-}$ (to produce Gd−Tex$^{\bullet+}$ and O_2) and TMQ$^{\bullet-}$ (to produce Gd−Tex$^{\bullet+}$ and TMQ), respectively. Equilibrium constants for the M-Tex^{2+} species reacting with $O_2^{\bullet-}$ and TMQ$^{\bullet-}$ were also determined from analysis of the absorption following establishment of the equilibrium. The resulting values for Gd−Tex^{2+} were found to be 6.8 and 10.7, respectively. From these equilibrium constants, the redox potential for the M−Tex^{2+}/M−Tex$^{\bullet+}$ couples at pH 8.5 were estimated and found to be ca. −110 mV vs NHE in the case of Gd−Tex^{2+}.

Introduction

The gadolinium(III) complex of the expanded porphyrin macrocycle texaphyrin, motexafin gadolinium (Gd−Tex^{2+}, Xcytrin), is currently in phase III US human clinical trials as a radiation sensitizer for the treatment of metastatic brain tumors. The mechanism by which Gd−Tex^{2+} increases the effectiveness of ionizing radiation is still under investigation.[1,2] A possible explanation for its activity is the finding that even in the absence of ionizing radiation Gd−Tex^{2+} produces H_2O_2, via initial production of $O_2^{\bullet-}$, in the presence of reducing metabolites, e.g., ascorbate.[2] Recently, the role of such reactive oxygen species (ROS) in modulating biological radiation response has attracted much interest. In principle, the oxidative stress caused by these species can enhance radiation response by increasing DNA damage, inhibiting DNA repair, or inducing apoptosis.[3−12] To understand better the chemical basis for reactive oxygen species formation in the presence of Gd−Tex^{2+}, superoxide anion was generated and its interaction with Gd−Tex^{2+} examined using pulsed radiolytic techniques. Briefly, it was found that in aqueous solution an equilibrium exists between $O_2^{\bullet-}$ and the four representative metallotexaphyrins tested (Figure 1), namely, Gd−Tex^{2+}, Lu−Tex^{2+}, Dy−Tex^{2+}, and Y−Tex^{2+} (eq 1). This was established on the basis of several measurements, including one that used 2,3,5-trimethyl-*p*-benzoquinone (TMQ) as a dioxygen surrogate. TMQ has a reduction potential similar to that of $O_2^{\bullet-}$ (−165 mV and −155 mV vs NHE at pH 7, respectively)[13] justifying its use in this regard. The use of this agent also allowed the reduction potentials for the M−Tex^{2+}/M−Tex$^{\bullet+}$ couples to be estimated more precisely.

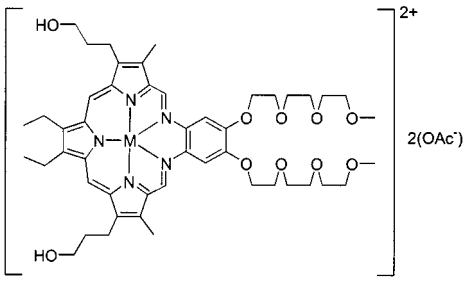

Figure 1. Structures of the complexes examined in this report.

$$O_2^{\bullet-} + M{-}Tex^{2+} \underset{k_{-1}}{\overset{K}{\underset{k_1}{\rightleftharpoons}}} M{-}Tex^{\bullet+} + O_2 \quad (1)$$

(M = Gd(III), Lu(III), Dy(III), and Y(III))

Experimental Section

Pulse radiolysis experiments were performed using a model TB-8/16-1S electron linear accelerator (Notre Dame Radiation Laboratory) producing 50 ns pulses of 8 MeV electrons. A complete description of the instrumental setup has been reported

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previously.[14,15] The dose per pulse was determined by potassium thiocyanate dosimetry, and was calculated to be 14 Gy, yielding total radical concentrations between 2 and 8 μM. For measurements under oxic conditions, aqueous buffer solutions (2.0 mM Na_2HPO_4) containing 2 vol % *tert*-butyl alcohol (pH 8.5) and 0−90 μM metallotexaphyrin were purged with a 30/70 oxygen/nitrogen mixture ($[O_2] \approx 390$ μM). The yield of reducing entities amounts, however, only to 50%. We selected *tert*-butyl alcohol rather than sodium formate (0.01 M) for the following reasons: (i) to avoid complexation of the texaphyrins by formate, a relatively strong axial ligand whose presence could alter the chemistry of the texaphyrin complexes, (ii) to retain the same experimental conditions as used in previous studies, and (iii) to guarantee clean radiolytic conditions. In this context it is important to note that the reactivity of the $(CH_3)_2C(OH)CH_2$-OO• radicals, for example, with ascorbate ion (2.1×10^6 M^{-1} s^{-1}) and TMPD (4.4×10^7 M^{-1} s^{-1}), is 3−4 orders of magnitude less than that of OH• radicals (ascorbate ion, 1.1×10^{10} M^{-1} s^{-1}; TMPD, 1.0×10^{10} M^{-1} s^{-1}).[16,17]

The above solutions were then irradiated, and the changes in the reduced texaphyrin (M−Tex$^{\bullet+}$) band at 830 nm and in the superoxide anion absorption band at 255 nm were measured over the course of 0−5 ms. Unfortunately, the latter wavelength, although giving rise to the greatest differences in optical signature as a function of changes in $O_2^{\bullet-}$ concentration, is "contaminated" by variations in "background" absorbances due to changes in [M−Tex$^{\bullet+}$]. A correction for this was made, as discussed in the results and discussion section. Aqueous solutions containing oxygen concentrations of ca. 1.3 mM and 280 μM (O_2 and air saturated, respectively) were also tested.

For measurements involving quinone reduction, an aqueous solution containing 2% *tert*-butyl alcohol and TMQ (500 μM) was purged with nitrogen. To this solution, aliquots of a 200 μM stock texaphyrin solution were added as needed. The 310 nm differential absorption was monitored following irradiation of solutions in which the metallotexaphyrin concentration ranged from ca. 0 to 120 μM, and the quinone concentration from ca. 500 to 200 μM.

The synthesis and characterization of the metallotexaphyrins tested (Gd−Tex^{2+}, Lu−Tex^{2+}, Dy−Tex^{2+}, and Y−Tex^{2+}) have previously been reported.[18] Unless otherwise indicated, all of the reagents employed in this work were purchased from Sigma-Aldrich and used without further purification. TMQ was synthesized from 2,3,6-trimethylphenol and peracetic acid as reported in the literature.[19]

Results and Discussion

Initial measurements to test for the production of superoxide, were designed such that a large excess of oxygen as compared to metallotexaphyrin was present at the time of irradiation. This was done since the rate of the observed reaction (vide infra) was found to depend on the concentration of both species. Designed in this way, our studies allowed for the selective production of superoxide radical anions via reaction of O_2 with the hydrated electrons as shown in eq 2.

$$e^-_{(aq)} + O_2 \rightarrow O_2^{\bullet-} \qquad (2)$$

Under these conditions, a reaction between the superoxide anion and the metallotexaphyrins of this study was observed to occur on the 0.5−1.0 ms time scale, as manifest by the increase in decay rate of the superoxide radical anion absorption at 255 nm ($V_{obs} = -d[O_2^{\bullet-}]/dt = k_{obs}[O_2^{\bullet-}]$) as a function of the metallotexaphyrin concentration. Under these conditions an absorption band at λ_{max} at 830 nm, characteristic of reduced metallotexaphyrin radical cations (M-Tex$^{\bullet+}$),[18] was also observed to grow in over the course of 1−1.5 ms. At longer times (i.e., up to 5 ms), the absorbance decay ascribed to the superoxide radical anions (i.e., that followed at 255 nm) was seen to level off, rather than to decay to zero, in the presence of metallotexaphyrin (Figure 2). This is not ascribed to disproportionation of $O_2^{\bullet-}$ ($k = 1 \times 10^5$ M^{-1} s^{-1})[20] since under the reaction conditions there is a large excess of M−Tex^{2+} as compared to $O_2^{\bullet-}$.

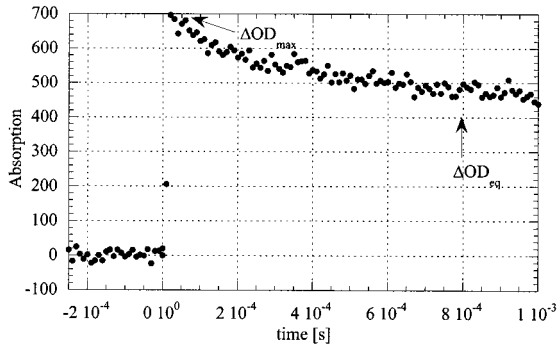

Figure 2. Time-trace recorded at 255 nm of an aqueous solution purged with a 30/70% O_2/N_2 mixture, and containing 70 μM Gd−Tex^{2+}, illustrating how ΔOD_{max} and ΔOD_{eq} were determined.

In contrast to what is seen at 255 nm in monitoring the changes in superoxide radical dynamics, the absorption profile of the singly reduced metallotexaphyrin species, monitored at 830 nm, was found to return to the baseline. Such a complete return to baseline also stands as a difference in what is observed when metallotexaphyrins are subject to reduction with hydrated electrons. Under these latter conditions, the singly reduced metallotexaphyrin was found to decay via protonation followed by disproportionation as well as via dimerization prior to protonation. This gives rise to species (i.e., M−TexH$^+$, M−TexH^{2+}, and $(M-Tex)_2H^{\bullet4+}$) that have distinctly different absorption spectra as compared to the unreacted metallotexaphyrins.[18]

Taken in concert, the above observations are consistent with the singly reduced metallotexaphyrin species (M−Tex$^{\bullet+}$), produced via the reaction of $O_2^{\bullet-}$ and M−Tex^{2+}, reacting with oxygen to regenerate M−Tex^{2+} (and produce $O_2^{\bullet-}$). If this reaction is a simple one-electron process, then reaction of singly reduced metallotexaphyrin would produce superoxide radical anions, and thus, the equilibrium depicted in eq 1 above would be occurring on the pulse radiolytic time scale.

To test the validity of this conclusion, the rate of superoxide absorbance decay was measured at various M−Tex^{2+} and O_2 concentrations. The data were then fit to the kinetic expression given in eq 3 (c.f. Supporting Information for derivation).

$$V_{obs}/[O_2] = k_1([M-Tex^{2+}]/[O_2]) + k_{-1} \qquad (3)$$

$$K = k_1/k_{-1} \qquad (4)$$

To the extent M−Tex$^{\bullet+}$ is being generated by the reaction of $O_2^{\bullet-}$ and M−Tex^{2+}, plots of $k_{obs}/[O_2]$ vs $[M-Tex^{2+}]/[O_2]$ should be linear and afford the rate constant for the forward reaction (k_1) as the slope, and the back reaction rate constant (k_{-1}) as the intercept. Here k_1 and k_{-1} are as defined in eq 1, and again assuming the single step reaction of eq 1, the equilibrium constant K can then be calculated from eq 4.

Separately, it should be possible to calculate the equilibrium constant directly from the equilibrium expression of eq 1.

Rewriting this latter in the form of eq 5, makes it apparent that a plot of $[O_2]/[M\text{-}Tex^{2+}]$ vs $[O_2^{\bullet-}]/[M\text{-}Tex^{\bullet+}]$ should yield K as the slope.

$$[O_2]/[M-Tex^{2+}] = K([O_2^{\bullet-}]/[M-Tex^{\bullet+}]) \quad (5)$$

Here, the ratio of $[O_2]/[M-Tex^{2+}]$ can be approximated from the reaction conditions employed (i.e., $[O_2] \approx [O_2]_0$ and $[M-Tex^{2+}] \approx [M-Tex^{2+}]_0$, where $[O_2]_0$ and $[M-Tex^{2+}]_0$ are the starting concentrations of those species). By contrast the ratio of radical concentrations is not set by the initial conditions. Rather, this critical parameter may be calculated from the absorption observed at equilibrium using eq 6, where A is the absorption measured at the equilibrium, and $A_{M-Tex}^{\bullet+}$ and $A_{O_2}^{\bullet-}$ are the 255 nm differential absorptions of the $M-Tex^{\bullet+}$ and $O_2^{\bullet-}$ radicals without the other species present. Thus, in practical terms the plot of $[O_2]/[M-Tex^{2+}]$ vs $[O_2^{\bullet-}]/[M-Tex^{\bullet+}]$ becomes one of $[O_2]/[M-Tex^{2+}]$ vs $(A - A_{M-Tex}^{\bullet+})/(A_{O_2}^{\bullet-} - A)$; it is this that yields K as the slope (eq 7).

$$[O_2^{\bullet-}]/[M-Tex^{\bullet+}] = (A - A_{M-Tex}^{\bullet+})/(A_{O_2}^{\bullet-} - A) \quad (6)$$

$$[O_2]/[M-Tex^{2+}] = K(A - A_{M-Tex}^{\bullet+})/(A_{O_2}^{\bullet-} - A) \quad (7)$$

While 255 nm is the wavelength at which the greatest change in optical signature as a function of $[O_2^{\bullet-}]$ is seen, the small magnitude of the signal coupled with the observation of a significant baseline drift, required a normalization of the observed absorption changes. This was done using eq 8, where ΔOD_{max} and ΔOD_{eq} are shown in Figure 2, $\Delta OD'_{eq}$ is the corrected differential absorption term we are seeking (eq 9), and $\Delta OD'$ is the background absorption calculated from the rate of reaction of $M-Tex^{2+}$ directly with $e^-_{(aq)}$ (eq 10).

$$(\Delta OD_{max})/(\Delta OD') = (\Delta OD_{eq})/(\Delta OD'_{eq}) \quad (8)$$

$$\Delta OD'_{eq} = A = \Delta OD_{eq}(\Delta OD'/\Delta OD_{max}) \quad (9)$$

$$\Delta OD' = A_{O_2}^{\bullet-}(a) + A_{M-Tex}^{\bullet+}(b) \quad (10)$$

The first of these equations was derived by recognizing the equivalence between A and $\Delta OD'_{eq}$. This allows eq 8 to be rewritten directly as eq 9. On the other hand, eq 10, used to calculate $\Delta OD'$, was obtained by adjusting the absorbance of $O_2^{\bullet-}$ and $M-Tex^{\bullet+}$ to account for the loss in $[M-Tex^{2+}]$ arising from direct reaction with $e^-_{(aq)}$. This was accomplished by using the known rate constants for reaction of $M-Tex^{2+}$ and O_2 with hydrated electrons,[18,21] affording ratio $b = [([M-Tex^{2+}]/[O_2])-(k_{M-Tex}^{2+}/k_{O2})]/[1 + ([M-Tex^{2+}]/[O_2])(k_{M-Tex}^{2+}/k_{O_2})]$, and $a = 1 - b$.

Assuming single step kinetics, the equilibrium constants (K) obtained under conditions of equilibrium (eq 7) and those calculated from eq 4, with k_1 and k_{-1} determined from the approach to the equilibrium according to eq 3, should afford similar values. Thus, to the extent that the K values determined from these two analyses match, it becomes reasonable to assume that the equilibrium of eq 1 is indeed fast on the pulse radiolytic time scale. Here, independent of other considerations it is important to appreciate that one practical limitation is that the reduction potential of the two compounds being tested (i.e., $O_2^{\bullet-}$ and $M-Tex^{\bullet+}$) will have to be within 150 mV of each other.[22] This is because the back reaction has to be fast enough to be observed on the time scale of pulse radiolysis.

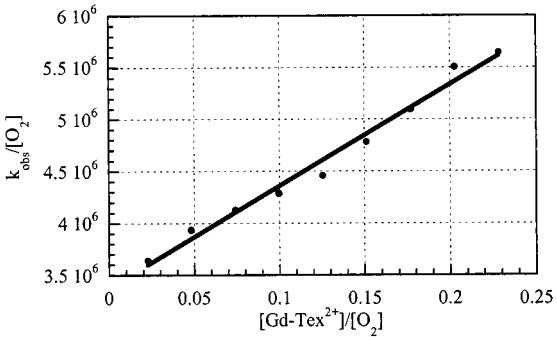

Figure 3. Kinetic plot of the approach to equilibrium for the reaction of $O_2^{\bullet-}$ and $Gd-Tex^{2+}$ in accord with eq 1 ($R^2 = 0.992$).

TABLE 1: Rate Constants and Equilibrium Constants for Equation 1 Obtained from Both Kinetic and Absorption-Based Equilibrium Analyses[a]

compound	kinetic k_1 [M^{-1} s^{-1}]	kinetic k_{-1} [M^{-1} s^{-1}]	kinetic equilibrium constants[b] K_{kin}	absorption equilibrium constants[c] K_{eq}
$Gd-Tex^{2+}$	9.8×10^6	3.4×10^6	2.9	6.8
$Lu-Tex^{2+}$	1.4×10^7	1.6×10^6	8.8	9.4
$Dy-Tex^{2+}$	1.3×10^7	4.3×10^6	3.0	7.2
$Y-Tex^{2+}$	7.5×10^6	3.2×10^6	2.3	10.9

[a] Estimated errors are ±15% and ±30% for K determined under kinetic and equilibrium conditions, respectively. [b] K of eq 1 measured under the kinetic conditions described in the text. [c] K of eq 1 measured under the absorption-monitored equilibrium conditions described in the text.

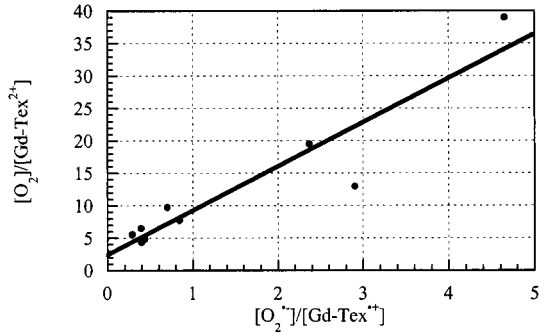

Figure 4. Plot of $[O_2]/[Gd-Tex^{2+}]$ vs $[O_2^{\bullet-}]/[Gd-Tex^{\bullet+}]$ determined from changes in absorption at 255 nm following a presumed establishment of equilibrium in accord with eq 1 ($R^2 = 0.937$). See text for details of data analysis.

Figure 3 shows the kinetic plot for the approach to the equilibrium in the reaction of $O_2^{\bullet-}$ and $Gd-Tex^{2+}$. This plot is clearly linear, and yields a k_1 of 9.81×10^6 M^{-1} s^{-1} and k_{-1} of 3.38×10^6 M^{-1} s^{-1}. Plots obtained for the other metallotexaphyrins tested displayed similar linearity. The resulting k_1 and k_{-1} values are presented in Table 1, and range from a maximum k_1 of 1.4×10^7 M^{-1} s^{-1} for $Lu-Tex^{2+}$ to 7.5×10^6 M^{-1} s^{-1} for $Y-Tex^{2+}$, and a k_{-1} high of 4.3×10^6 M^{-1} s^{-1} for $Dy-Tex^{2+}$ and low of 1.6×10^6 M^{-1} s^{-1} for $Lu-Tex^{2+}$.

Analysis of the equilibrium reaction for $Gd-Tex^{2+}$ in accord with eq 7 (Figure 4) also gave rise to a linear plot from which a K of 6.8 was obtained. Similar results were obtained for the other $M-Tex^{2+}$ species tested.

Although the kinetics and absorption analyses are different, they both yielded linear plots for all the metallotexaphyrins tested. They also gave equilibrium constants that were within a factor of 5 of one another (Table 1). That there is this level of correspondence is gratifying. On the other hand the obvious disparities, particularly apparent in the case of $Gd-Tex^{2+}$, $Dy-$

Tex^{2+}, and $Y-Tex^{2+}$, warrants consideration. There is an inherent 10% error in the pulse radiolytic kinetics measurements, which through the propagation of error leads to an error of ca. 15% in *K* values determined from the ratio of k_1 and k_{-1}. For *K* determined under conditions of equilibrium, there is an inherent error of ca. 30% reflecting the 10% error inherent in each of the absorption analyses. The key point, however, is that, even accounting for these error limitations, there are clear differences in the *K* values measured under kinetic, as opposed to thermodynamic, conditions at least for the Gd(III), Dy(III), and Y(III) texaphyrin complexes. This discrepancy could have its origins in the fact that the singly reduced species of these three metallotexaphyrins are protonated at very rapid rates, i.e., rate constants on the order of 9×10^8 M^{-1} s^{-1}.[18] It is thus possible that some of the singly reduced texaphyrin species becomes protonated before, and instead of, reacting with O_2.[23,24] To the extent such a competition occurs, the approach to the equilibrium would no longer be completely pure. This would affect *K* values determined under kinetic conditions in such a way that the derived K_{eq} values would appear smaller. This is because protonation effectively removes $Gd-Tex^{\bullet+}$ from circulation thereby driving the equilibrium of eq 1 to the right with an attendant increase in k_{obs} (see Supporting Information). In any event subject to this potential limitation in accuracy, it is clear that $Gd-Tex^{\bullet+}$ reacts with O_2 to produce $O_2^{\bullet-}$ effectively, and likely does so with a rate constant on the order of 10^6-10^7 M^{-1} s^{-1}.

In previous work involving electrochemical analyses, an $E_{1/2}$ values of 80 mV vs NHE and −41 mV vs NHE were determined for $Gd-Tex^{2+}$ in aqueous media and DMF, respectively.[1,18] The present analyses provide an alternative means of determining this key value. In particular, since the redox potential of the $O_2/O_2^{\bullet-}$ couple is known, the redox potential of the $M-Tex^{2+}/M-Tex^{\bullet+}$ couple can be determined from the equilibrium constants using eq 11 (R = universal gas constant (8.3145 J/molK), T = temperature (298 K), n = number of electrons involved (1), F = Faraday's constant (9.6485×10^4 C/mol), and $E^\circ_{1/2}(O_2/O_2^{\bullet-})$ in saturated aqueous solution = −155 mV at pH 7[13]). The value calculated in this way, −110 mV vs NHE, compares favorably with the value recorded previously in DMF, but differs substantially from that recorded earlier in aqueous media. Whether this latter discrepancy reflects simple differences in pH (7.0 previously, 8.5 currently) or more substantive effects is not know at present and awaits the outcome of more detailed electrochemical analyses currently ongoing.

$$\Delta G^\circ = -RT \ln K = -nF[E^\circ_{1/2}(O_2/O_2^{\bullet-}) - E^\circ_{1/2}(M-Tex^{2+}/M-Tex^{\bullet+})] \quad (11)$$

Similar analyses were carried out for $Y-Tex^{2+}$, $Dy-Tex^{2+}$, and $Lu-Tex^{2+}$ and yielded the $E_{1/2}$ values collected in Table 2. While the reduction potentials for these latter species have yet to be determined in aqueous media, the reduction potentials calculated for these complexes via this indirect approach, are all found to be similar to, but shifted anodically, those obtained in DMF using cyclic voltammetry.[18]

Problems with baseline drift and the low signal-to-noise ratio for studies involving $O_2^{\bullet-}$, led us to carry out indirect analyses of $E_{1/2}$ using an alternative method in which trimethyl-*p*-benzoquinone (TMQ) was used as a dioxygen surrogate (eq 12). This quinone was chosen as its reduced species displays a large differential absorption change at 310 nm, and its reduction potential is −165 mV vs NHE in aqueous media, pH 7,[13] which is within 75−110 mV of the various $M-Tex^{2+}/M-Tex^{\bullet+}$ couples.

TABLE 2: Reduction Potentials (vs NHE) for the $M-Tex^{2+}/M-Tex^{\bullet+}$ Couple at pH 8.5, Aqeuous Media, Calculated from the Equilibrium Constants Determined under Kinetic and Equilibrium Conditions, Respectively, and Determined by Cyclic Voltammetry in DMF[a]

compound	$E^\circ_{1/2}$ calculated from K_{kin} ($M-Tex^{2+}/M-Tex^{\bullet+}$) [mV]	$E^\circ_{1/2}$ calculated from K_{eq} ($M-Tex^{2+}/M-Tex^{\bullet+}$) [mV]	$E^\circ_{1/2}$ determined in DMF[16] ($M-Tex^{2+}/M-Tex^{\bullet+}$) [mV]
$Gd-Tex^{2+}$	−128	−106	−41
$Lu-Tex^{2+}$	−99	−97	−44
$Dy-Tex^{2+}$	−127	−104	−45
$Y-Tex^{2+}$	−134	−94	−64

[a] Estimated errors are ±15% and ±30% for $E_{1/2}$ determined from these two sets of equilibrium constants, respectively.

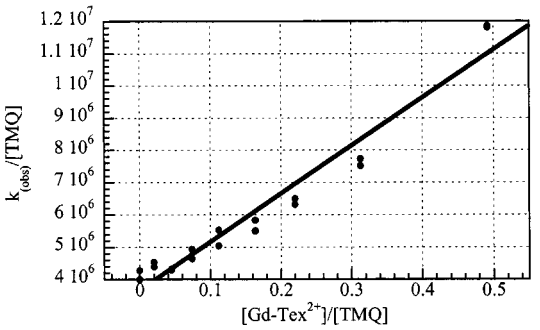

Figure 5. Kinetic plot of the approach to equilibrium between $TMQ^{\bullet-}$ and $Gd-Tex^{2+}$ in accord with eq 12 ($R^2 = 0.976$).

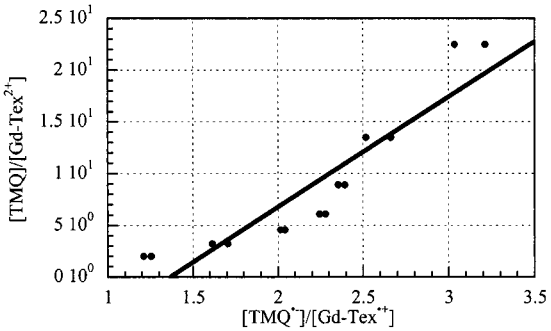

Figure 6. Plot of $^{-2+}$] vs $[TMQ^{\bullet-}]/[Gd-Tex^{\bullet+}]$ determined from the changes in absorption at 310 nm following a presumed establishment of equilibrium in accord with eq 12 ($R^2 = 0.917$).

$$TMQ^{\bullet-} + M-Tex^{2+} \underset{k_{-1}}{\overset{K}{\rightleftharpoons}} M-Tex^{\bullet+} + TMQ \quad (12)$$

As illustrated for the case of $Gd-Tex^{2+}$ (Figures 5 and 6), kinetic and absorption plots from experiments carried out using this quinone proved linear. This allowed two sets of *K* values to be calculated (Table 3). Again, these proved to be in reasonably good agreement, especially when complications due to rapid protonation rate are taken into account.

On the basis of the above *K* values redox potentials could be calculated. The resulting values, collected in Table 4, range between −104 and −139 mV vs NHE. They are thus all in excellent agreement with those calculated from the superoxide data (Table 2).

In summary we have put forward data that supports the contention that all of the prototypic texaphyrin complexes tested, namely $Y-Tex^{2+}$, $Dy-Tex^{2+}$, $Gd-Tex^{2+}$, and $Lu-Tex^{2+}$, participate in an equilibrium (eq 1) with superoxide radical anion on the pulse radiolytic time scale. Analysis of the approach to

TABLE 3: Rate and Equilibrium Constants Obtained from Both Kinetic and Absorption-Based Equilibrium Analyses Using TMQ as a Surrogate for Dioxygen

compound	kinetic k_1 [M^{-1} s^{-1}]	kinetic k_{-1} [M^{-1} s^{-1}]	kinetic equilibrium constant[a] K_{kin}	absorption equilibrium constant[c] K_{eq}
Gd−Tex^{2+}	1.5×10^7	3.7×10^6	4.1	10.7
Lu−Tex^{2+}	8.9×10^6	1.0×10^6	8.6	8.1
Dy−Tex^{2+}	1.7×10^7	3.3×10^6	5.2	5.1
Y−Tex^{2+}	1.1×10^7	3.9×10^6	2.8	3.7

[a] Estimated errors are ±15% and ±10% for K determined under kinetic and equilibrium conditions, respectively. [b] K of eq 12 measured under the kinetic conditions described in the text. [c] K of eq 12 measured under the absorption-monitored equilibrium conditions described in the text.

TABLE 4: Reduction Potentials (vs NHE) for the M−Tex^{2+}/M−$Tex^{\bullet+}$ Couple at pH 8.5, Aqeuous Media, Calculated from the Equilibrium Constants Determined under Kinetic and Equilibrium Conditions, Respectively, Using TMQ[a]

compound	$E°_{1/2}$ calculated from K_{kin} (M−Tex^{2+}/M−$Tex^{\bullet+}$) [mV]	$E°_{1/2}$ calculated from K_{eq} (M−Tex^{2+}/M−$Tex^{\bullet+}$) [mV]
Gd−Tex^{2+}	−129	−104
Lu−Tex^{2+}	−110	−111
Dy−Tex^{2+}	−123	−123
Y−Tex^{2+}	−139	−131

[a] Estimated errors are ±15% and ±10% for $E_{1/2}$ determined from these two sets of equilibrium constants, respectively.

equilibrium as well as separate analyses carried out after equilibrium was established, afforded K values for eq 1 that match each other within a factor of 5. These K values, which fall within the range of 2.3 and 10.9, also allow the reduction potentials of the M−Tex^{2+}/M−$Tex^{\bullet+}$ couples to be calculated under the conditions of the pulse radiolytic experiments (aqueous, pH 8.5). Confirmatory studies, using TMQ as a dioxygen surrogate, were also performed. These latter experiments afforded K and $E_{1/2}$ values that match those obtained in the presence of $O_2^{\bullet-}$.

The present results thus provide important support for ongoing mechanistic analyses. In particular, the fact that singly reduced texaphyrins, M−$Tex^{\bullet+}$, are capable of producing superoxide radical anions in the presence of O_2 is significant. Increases in $O_2^{\bullet-}$, and H_2O_2 derived therefrom, have been shown to induce programmed cell death (apoptosis), especially when produced in the mitochondrion.[4−12] This leads to the consideration that Xcytrin (Gd−Tex^{2+}), an agent known to localize in tumors very efficiently, mediates its radiation sensitizing effect in whole, or in part by the site-selective production of $O_2^{\bullet-}$ or H_2O_2, with the critical reduced Gd−$Tex^{\bullet+}$ species being produced either by direct electron capture under conditions of exposure to ionizing radiation[18] or via reaction with endogenous reducing agents such as ascorbate anion.[2] Under the latter conditions, a process of futile redox cycling could be set up wherein the fast equilibrium of eq 1 is expected to play a critical role.[25]

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Supporting Information Available: Derivation of eqs 3 and 6 and discussion of the effect of protonation upon the equilibrium constant K_{kin}. Calculation of the intracellular concentration of O_2 under the experimental conditions, normal, and hypoxic oxygen tension. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) Assuming an [O_2] of 380 μM (cf. Supporting Information) and rate constants for reaction with O_2 and H^+ of 3.4×10^6 M^{-1} s^{-1} and 1.0×10^9 M^{-1} s^{-1},[16] respectively, a branching ratio of quenching by O_2 vs protonation of 0.2% is calculated at pH 8.5 for Gd−Tex^{2+}. Complexities due to this kind of competition constitute a known problem. For a mathematical treatment see: Moenig, J.; Goslich, R.; Asmus, K.-D. *Ber. Bunsen-Ges. Phys. Chem.* **1986**, *90*, 115−121.

(24) Further support for the contention that protonation can compete with superoxide production is the finding that no reaction in accord with eq 1 is seen at pH 3.8.

(25) The conclusion, that Gd−$Tex^{\bullet+}$ produces superoxide in vivo, is predicated on the assumption that Gd−$Tex^{\bullet+}$ is long-lived enough to react with O_2 under physiological conditions. Since the rate constant for protonation of Gd− $Tex^{\bullet+}$ is very rapid (1×10^9 M^{-1} s^{-1}),[18] it is possible that a competition between protonation and $O_2^{\bullet-}$ formation exists under physiological conditions (pH 7.4). Taking the partial pressure of free O_2 within normal and hypoxic cells as 30 and 3 Torr, respectively,[26] and using Henry's law it is possible to determine the concentration of free O_2 within these cells as 50 and 5 μM, respectively (cf. Supporting Information). With a free proton concentration at physiological pH (7.4) of ca. 4.0×10^{-8} M (0.04 μM) and pseudo first-order rate constants for the reaction of Gd−$Tex^{\bullet+}$ with O_2 and H^+, of 3.4×10^6 M^{-1} s^{-1} and 1.0×10^9 M^{-1} s^{-1},[16] respectively, the rates of reaction of Gd−$Tex^{\bullet+}$ with O_2 under normal and hypoxic conditions are calculated to be 170 s^{-1} and 17 s^{-1}, respectively, while the rate of protonation of Gd−$Tex^{\bullet+}$ at pH 7.4 is calculated to be 40 s^{-1}. Thus, under normal conditions, the formation of superoxide anions is ca. 4 times faster than protonation, while under hypoxic conditions protonation is expected to be twice as fast as superoxide anion formation. Since the solubility of O_2 in salt and protein rich cellular media is likely to be higher than that in pure water, this ratio should be shifted in favor of $O_2^{\bullet-}$ formation within the cell. If the solubility of O_2 in intracellular medium is estimated at 5 mM/atm, an intermediate value between the 1.27 mM/

atm of pure water and 9.88 mM/atm of toluene,[27] then the concentration of O_2 in hypoxic cells is ca. 20 μM. Under these conditions, reaction of Gd−Tex$^{\bullet+}$ with O_2 to generate $O_2^{\bullet-}$ becomes favored over protonation by a factor of 1.5. In the event that the protonation is reversible, this ratio would be further enhanced. On the other hand, to the extent that other species, including endogenous phosphate entities, or even H_2O, could serve as proton donors, this ratio would be diminished. At present, however, there is no evidence that these latter species function in this way.

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