

plings. Thus, the J_{CC}/J_{HH} ratio should be in the range of $+(0.4)^2$ to $+(0.7)^2$, or $+0.16$ to $+0.49$ (because $J_{CC}/J_{HH} = J_{CC}/J_{CH} \times J_{CH}/J_{HH}$). Indeed, the J_{CC}/J_{HH} ratio (for J_{14} of **1** and J_{HH} of acetylene⁹) is $+1.84/+9.53 = +0.2$.

Thus, in the acetylenic compound **1**, the J_{CC} values do compare with geometrically equivalent J_{CH} and J_{HH} values in the manner that J_{CH} values compare with geometrically equivalent J_{HH} values. Evidence therefore continues to accumulate that carbon-13 behaves as proton in nmr couplings and that couplings involving carbon-13 have similar mechanisms to those involving proton.

Work is continuing to determine J_{CC}/J_{CH} , including signs, for multiply labeled olefinic and aliphatic compounds.

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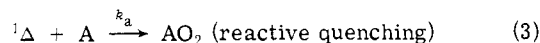
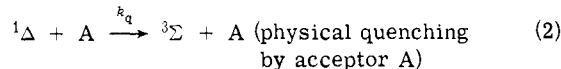
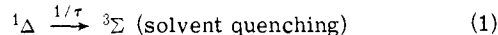
Comment Regarding the Rate Constant for the Reaction between 1,3-Diphenylisobenzofuran and Singlet Oxygen

Sir:

Recently Matheson, *et al.*, stated¹ that the rate constant for reaction between 1,3-diphenylisobenzofuran (DPBF) and singlet oxygen ($^1\Delta$) reported by ourselves,^{2,3} and oth-

ers,⁴ is an order of magnitude too high and primarily reflects physical quenching of $^1\Delta$ by DPBF rather than reactive quenching. This assertion is supported neither by our own laser photolysis data nor by data from several other laboratories.

While DPBF no doubt physically quenches $^1\Delta$ to some extent, an analysis of photooxidation efficiencies leads us to rule out this process as a major decay pathway. Under the conditions of our experiments³ the possible routes for $^1\Delta$ decay are



where A is DPBF in the present case. Since [A] does not change too drastically following a laser pulse, it is possible to derive the following expression to explain the observed bleaching of DPBF

$$[AO_2]_{t=\infty} - [AO_2]_t \approx \frac{k_a[A][^1\Delta]_{t=0}}{1/\tau + (k_q + k_a)[A]} e^{-\{1/\tau + (k_q + k_a)[A]\}t} \quad (4)$$

where $[AO_2]_{t=\infty}$ is the concentration of products after complete decay of $^1\Delta$ and $[^1\Delta]_{t=0}$ is the concentration produced by the laser pulse. Substituting $[AO_2] = 0$ at $t = 0$ and rearranging gives

$$[^1\Delta]_{t=0} = [AO_2]_{t=\infty} \frac{1/\tau + (k_q + k_a)[A]}{k_a[A]} \quad (5)$$

In an earlier report³ we assumed that the observed DPBF quenching constant, $(k_q + k_a)$, was approximately equal to k_a itself. Using the measured values of τ , k_a , and $[AO_2]_{t=\infty}$ in methanol, we calculated that under suitable conditions (see Results, section 3 in ref 3) $[^1\Delta]_{t=0}$ was equal to $90 \pm 10\%$ of the concentration of sensitizer (Methylene Blue) triplets produced by a pulse. If, as Matheson and Lee suggest, $k_a \approx 0.1(k_q + k_a)$, the quantum efficiency of $^1\Delta$ production from Methylene Blue Triplets would have to be ~ 9.0 . In order not to exceed the generally accepted maximum quantum efficiency of 1.0, k_q must in fact be $\leq 0.1k_a$ justifying our above assumption.

The photolysis data of Adams and Wilkinson also support this conclusion.⁵ Referring to Figure 3 in ref 5, it is evident that essentially all of the initial level of $5 \times 10^{-5} M$ DPBF is bleached in a single laser pulse. The concentration of $^1\Delta$ produced by the pulse cannot exceed $10^{-4} M$, the concentration of Methylene Blue sensitizer. If nine molecules of $^1\Delta$ were indeed quenched by DPBF for each which reacts, then at most only $1 \times 10^{-5} M$ DPBF could be bleached. This limit is further reduced if competition by solvent quenching is included in the analysis.

Usui has measured quantum yields of photooxidation of DPBF (number of molecules oxidized per photon absorbed by Methylene Blue) in methanol.⁶ Values near unity were obtained at DPBF concentrations as low as $\sim 10^{-4} M$. Again, if $k_a \approx 0.1(k_q + k_a)$, then the quantum yield could never exceed 0.1.

Olmsted and Akashah⁷ have used their quantum efficiency data and our τ value in methanol (which is accurate regardless of the relative magnitudes of k_q and k_a) to calculate a value of k_a for DPBF of $6.13 \times 10^8 M^{-1} \text{ sec}^{-1}$ quite close to our value of $8 \times 10^8 M^{-2} \text{ sec}^{-1}$. Reactive and physical quenching are unambiguous here as in the other efficiency measurements.

The reason why Matheson and Lee obtain a lower value of k_a is not apparent. Although their data was obtained in Freon 11 rather than methanol, this should not be important since k_a is not very solvent dependent.³ Possibly the difficulty lies in their determination of the number of singlet oxygen molecules produced by direct laser excitation. It is interesting to note that the rate constant for quenching of $^1\Delta$ by β -carotene reported by Matheson and Lee is about a factor of 10 less than values obtained by Foote, *et al.*,⁸ Farmilo and Wilkinson,⁹ and ourselves.³

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Extrusion of Fe_2S_2^* and Fe_4S_4^* Cores from the Active Sites of Ferredoxin Proteins

Sir:

We have recently demonstrated that polynuclear cluster complexes of general formulation $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ and $[\text{Fe}_2\text{S}_2(\text{SR})_4]^{2-}$ ($\text{R} = \text{alkyl}$), which serve as synthetic analogs^{2,4-9} of the active sites of oxidized ferredoxin proteins¹⁰ (Fd_{ox}), undergo facile thiolate substitution reactions. These reactions, effected by the addition of thiols $\text{R}'\text{SH}$ to solutions of cluster complexes at ambient temperature, result in the transfer of Fe_4S_4^* and Fe_2S_2^* cores from one ligand environment to another with little or no decomposition and no important changes in core structure.^{3,5} Among these reactions the conversion of Fe_4S_4 -glycyl-L-cysteinylglycyl oligopeptide complexes⁴ and $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$ ($\text{S}_2\text{-}o\text{-xyl} = o\text{-xylene-}\alpha,\alpha'\text{-dithiolate}$) with benzenethiol to $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$ and $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$, respectively, offer clear precedents for the extrusion of core units from holoproteins in the form of their arylthiolate derivatives, reaction 1, in which the two known types of active sites with $n > 1$ ¹⁰ are indicated. From experiments based on previous work,¹⁻⁴ we report here the successful extrusions of

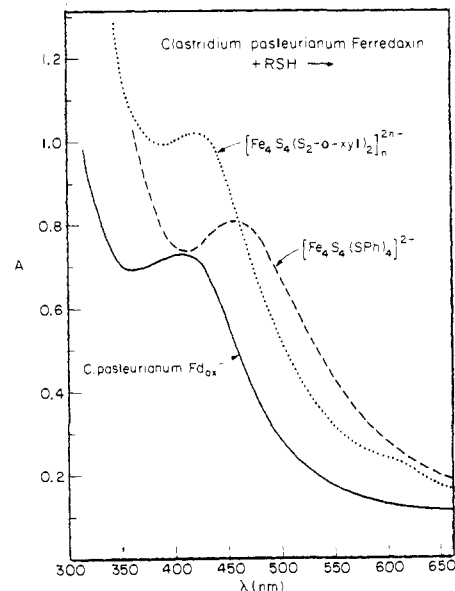
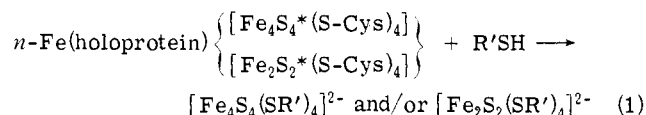


Figure 1. Spectrophotometric demonstration of two extrusion reactions of 8-Fd_{ox} in 80% DMSO. Product complexes were identified spectrally.²

the Fe_4S_4^* core of *Clostridium pasteurianum* ferredoxin (8-Fd_{ox}) and the Fe_2S_2^* core of the algal protein *Spirulina maxima* ferredoxin (2-Fd_{ox}).¹¹ In complementary and detailed studies Bale and Orme-Johnson¹² have demonstrated core extrusions from a number of other Fe-S proteins.



The utility of various thiols in extrusion reactions, which were monitored spectrophotometrically (Figures 1 and 2), has been examined; experimental conditions and results are given in Table I. In the 80% DMSO reaction medium employed the proteins are expected to be unfolded to a considerable extent,^{13,14} thereby allowing easier access of the thiol to the active site at which, extrapolating from kinetic studies of synthetic complexes,¹⁵ protonation of coordinated S-Cys by $\text{R}'\text{SH}$ initiates the substitution process. The shift of the visible band in the 8-Fd_{ox} spectrum from 390 nm in aqueous solution to 410 nm in 80% DMSO places it in the range of Fe_4S_4 -peptide complexes in this solvent (404–409 nm), indicating significant denaturation.⁴ Treatment of this protein with excess benzenethiol affords clean extrusion of the active site core in the form of thoroughly characterized $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$,^{2,3,7} readily distinguished from the protein by its band at 458 nm² (Figure 1). Similarly, reaction of 2-Fd_{ox} with benzenethiol and $o\text{-xyl}(\text{SH})_2$ leads to extrusion of the Fe_2S_2^* core as its $[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$ and $[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$ derivatives, respectively, whose spectra (Figure 2)

Table I. Results of Extrusion Reactions of Fd_{ox} Proteins with Thiols^a

Protein	R'SH	<i>n</i> -fold excess ^b	Product	λ_{max} , nm (ϵ , M)	% conversion ^c
8-Fd _{ox} ^c	PhSH	35	$[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{2-}$	458 (17,600)	>95
2-Fd _{ox} ^d	PhSH	40	$[\text{Fe}_2\text{S}_2(\text{SPh})_4]^{2-}$	490 (11,000)	>95
8-Fd _{ox}	$o\text{-xyl}(\text{SH})_2$	120	$[\text{Fe}_4\text{S}_4(\text{S}_2\text{-}o\text{-xyl})_2]^{2n-}$	419 (22,300)	>95
2-Fd _{ox}	$o\text{-xyl}(\text{SH})_2$	30	$[\text{Fe}_2\text{S}_2(\text{S}_2\text{-}o\text{-xyl})_2]^{2-}$	417 (11,200), 450 (sh), 590 (5000)	>95
8-Fd _{ox}	$\text{HSCH}_2\text{CH}_2\text{SH}$	60	$[\text{Fe}_2(\text{edt})_4]^{2-}$	367 (26,300), 522 (8000)	~75
2-Fd _{ox}	$\text{HSCH}_2\text{CH}_2\text{SH}$	120	$[\text{Fe}_2(\text{edt})_4]^{2-}$	Same	>90

^a Conditions: anaerobic; 4/1 v/v DMSO/H₂O, aqueous component pH 8.5 (0.02 M Tris buffer); 30 min reaction time at ~25°. ^b Maximum amount of thiol for indicated conversion under stated conditions; *n*-fold excess = 8*n* and 4*n* mol R'SH for 8-Fd_{ox} and 2-Fd_{ox}, respectively. ^c *C. pasteurianum*. ^d *Sp. maxima*. ^e Determined spectrophotometrically using ϵ data for proteins and complexes measured separately; per mole of protein.