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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Received August 26, 1974

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

Sir:

We have recently demonstrated that polynuclear clear ter complexes of general formulation $[Fe_4S_4(SR)_4]^{2-}$ [1-4] and $[Fe_2S_2(SR)_4]^{2-}$ [5] (R = alkyl), which serve as synthetic analogs[2,4-9] of the active sites of oxidized ferredoxin proteins[10] (Fd_{ox}), undergo facile thiolate substitution reactions. These reactions, effected by the addition of thiols R'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 complexes[4] and $[Fe_2S_2(S_2$-*o*-xyl$)_2]^{2-}$ [5] (S_2-*o*-xyl = *o*-xylene-α,α'-dithiolate) with benzenethiol to $[Fe_4S_4(SPh)_4]^{2-}$ and $[Fe_2S_2(SPh)_4]^{2-}$, 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 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-Johnson[12] have demonstrated core extrusions from a number of other Fe-S proteins.

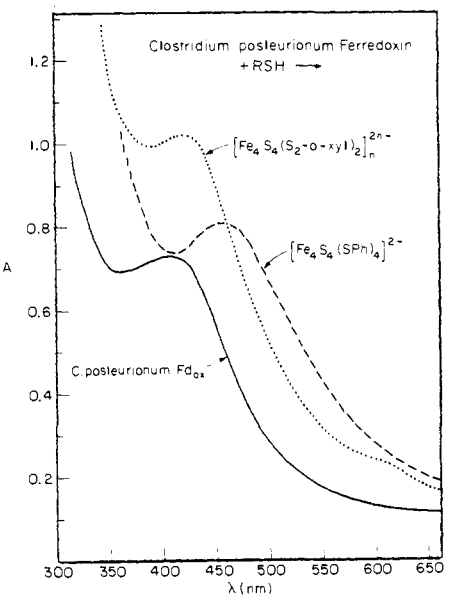

Figure 1. Spectrophotometric demonstration of two extrusion reactions of 8-Fd_{ox} in 80% DMSO. Product complexes were identified spectrally.[2]

$$n\text{-Fe(holoprotein)}\begin{Bmatrix}[Fe_4S_4^*(S\text{-Cys})_4]\\ [Fe_2S_2^*(S\text{-Cys})_4]\end{Bmatrix} + R'SH \longrightarrow [Fe_4S_4(SR')_4]^{2-} \text{ and/or } [Fe_2S_2(SR')_4]^{2-} \quad (1)$$

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 R'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 $[Fe_4S_4(SPh)_4]^{2-}$,[2,3,7] readily distinguished from the protein by its band at 458 nm[2] (Figure 1). Similarly, reaction of 2-Fd_{ox} with benzenethiol and *o*-xyl$(SH)_2$ leads to extrusion of the Fe_2S_2* core as its $[Fe_2S_2(SPh)_4]^{2-}$ and $[Fe_2S_2(S_2$-*o*-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	*n*-fold excess[b]	Product	λ_{max}, nm (ϵ_M)	% conversion[e]
8-Fd_{ox}[c]	PhSH	35	$[Fe_4S_4(SPh)_4]^{2-}$	458 (17,600)	>95
2-Fd_{ox}[d]	PhSH	40	$[Fe_2S_2(SPh)_4]^{2-}$	490 (11,000)	>95
8-Fd_{ox}	*o*-xyl$(SH)_2$	120	$[Fe_4S_4(S_2\text{-}o\text{-xyl})_2]_n^{2n-}$	419 (22,300)	>95
2-Fd_{ox}	*o*-xyl$(SH)_2$	30	$[Fe_2S_2(S_2\text{-}o\text{-xyl})_2]^{2-}$	417 (11,200), 450 (sh), 590 (5000)	>95
8-Fd_{ox}	$HSCH_2CH_2SH$	60	$[Fe_2(edt)_4]^{2-}$	367 (26,300), 522 (8000)	~75
2-Fd_{ox}	$HSCH_2CH_2SH$	120	$[Fe_2(edt)_4]^{2-}$	Same	>90

[a] Conditions: anaerobic; 4/1 v/v DMSO/H_2O, 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. pasteuranum*. [d] *Sp. maxima*. [e] Determined spectrophotometrically using ϵ data for proteins and complexes measured separately; per mole of protein.

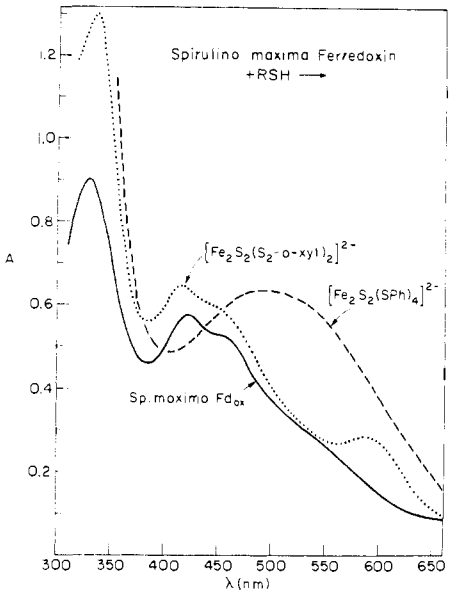

Figure 2. Spectrophotometric demonstration of two extrusion reactions of 2-Fd_{ox} in 80% DMSO. Product complexes were identified spectrally.[5,8]

are different from that of the protein and $[Fe_4S_4(SPh)_4]^{2-}$, and the same as those of the isolated complexes[5,8] measured separately.

With the demonstration of extrusion reactions of relatively simple proteins in hand, one of the potentially significant applications of reaction 1 would involve extension to complex Fe-S proteins and enzymes[10] in which the organization of active sites into 2-Fe and 4-Fe types cannot be satisfactorily established by spectroscopic methods. Several observations bearing on this point are noted. Experimental conditions for 2-Fd_{ox} extrusions must be carefully controlled. Preliminary kinetic measurements have shown that the dimer→tetramer conversion, reaction 2, is essentially

$$2[Fe_2S_2(SPh)_4]^{2-} \longrightarrow [Fe_4S_4(SPh)_4]^{2-} + PhSSPh + 2PhS^- \quad (2)$$

quantitative in 80% DMSO. Rates are dependent upon the pH of the aqueous component as the following dimer half-lives indicate: pH 8.5, 80 min; pH 7.9, 30 min (25°, dimer concentration *ca.* 10^{-4} *M*). The reaction is further retarded by excess thiol, such that, under the conditions employed, no significant amount of tetramer resulted. Reaction 2 must obviously be suppressed in order to prevent incorrect identification of the protein active site type when liberated as its arylthiolate derivative. A search has been made for thiol reagents capable of specific core extrusions. Although *o*-xyl$(SH)_2$ forms the stable binuclear complex $[Fe_2S_2(S_2\text{-}o\text{-}xyl)_2]^{2-}$, resistant to dimerization under extrusion conditions, its reaction with 8-Fd_{ox} and also $[Fe_4S_4(SEt)_4]^{2-}$ yields a product whose spectrum (Figure 1) indicates formation of an alkylthiolate tetramer,[2] presumably an oligomer of tetramers, $[Fe_4S_4(S_2\text{-}o\text{-}xyl)_2]_n^{2n-}$.[16] In contrast to the reactions with benzenethiol and *o*-xyl$(SH)_2$, treatment of both proteins with ethane-1,2-dithiol does not result in intact core extrusion. Instead the very stable *sulfide-free* dimer $[Fe_2(edt)_4]^{2-}$, previously characterized,[17] is obtained. The core structure of 8-Fd_{ox} is thus degraded, with the product complex obtained in reduced yield. With 2-Fd_{ox} the reaction proceeds through an unidentified intermediate species (λ_{max} 590 nm) and affords the dimer in high yield.

While no specific core extrusion reagents have been found, both benzenethiol and *o*-xyl$(SH)_2$ are capable of high-yield intact core removal from proteins in the form of spectrally distinguishable 2-Fe and 4-Fe complexes (Figure 1 and 2). Further, the electrochemical properties[5] of, *e.g.*, $[Fe_4S_4(SPh)_4]^{2-}$ and $[Fe_2S_2(SPh)_4]^{2-}$, are sufficiently different to permit analysis of solutions containing both species which might result from extrusion of a complex Fe-S protein. The use of the extrusion method to identify active sites in such molecules is under active investigation.[18] Any further elaboration of this method, especially with regard to the development of thiol reagents which afford protein core derivatives with more distinct or intense spectral features than those presented here, should be guided by the following observation. With all thiols thus far examined the reactions of the (readily accessible[5,7]) synthetic analogs $[Fe_2S_2(SR)_4]^{2-}$ and $[Fe_4S_4(SR)_4]^{2-}$ exactly parallel those of 2-Fd_{ox} and 8-Fd_{ox} proteins, respectively.

Acknowledgment. This research was supported at M.I.T. by National Institutes of Health Grant GP-19298. We thank R. Bare for experimental assistance.

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Received October 25, 1974

Dehydrations with *N*-Methyl-*N,N'*-di-*tert*-butylcarbodiimidium Ion

Sir:

In view of the broad utility of N,N'-disubstituted carbodiimides (**1**) for effecting molecular dehydrations,[1] it becomes of interest to evaluate the *N,N,N'*-trialkylcarbodi-

$$RN{=}C{=}NR \qquad RN{=}C{=}\overset{+}{N}(R')R$$
1 **2**