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Evidence for the existence of [2Fe-2S] as well as [4Fe-4S] clusters among F_A, F_B and F_X. Implications for the structure of the Photosystem I reaction center

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Core extrusion of the bound iron-sulfur centers from spinach Photosystem I showed the presence of [2Fe-2S] clusters as well as [4Fe-4S] clusters among F_A, F_B and F_X. Extrusion of the iron-sulfur ensemble was not quantitative; however, the presence of [2Fe-2S] clusters correlated with higher concentration of unfolding solvent. Since F_X is highly resistant to denaturation, and since F_A and F_B are known to contain [4Fe-4S] clusters, the [2Fe-2S] clusters are assigned to F_X. The presence of [2Fe-2S] clusters in Photosystem I has significance in the structure and organization of F_X on the reaction center. Since four cysteinyl ligands are assumed to hold an iron-sulfur cluster, a Photosystem I subunit may consist of two approx. 64-kDa proteins bridged by a single [2Fe-2S] cluster. The complete reaction center would consist of two subunits positioned so that two [2Fe-2S] clusters are in magnetic interaction, thereby constituting F_X.

Biochemical and biophysical studies have shown that a minimum of six membrane-bound components participate in photochemical charge separation in Photosystem I: an electron donor (P-700), a primary electron acceptor (A_0), an intermediate electron acceptor A_1, and three membrane-bound iron-sulfur centers (F_X, F_B and F_A). The chemical identities of these components are largely known: P-700 is thought to be a chlorophyll *a* dimer; A_1 has been identified as a phylloquinone; and F_X, F_B and F_A are iron-sulfur centers (see Refs. 1–3 for review).

There are two likely candidates for the type of iron-sulfur centers in Photosystem I: [2Fe-2S] clusters and [4Fe-4S] clusters. Results from methods used to identify the iron-sulfur clusters in Photosystem I include: (1) ESR spectroscopy in 80% DMSO, which indicates that F_A and F_B are [4Fe-4S] clusters [4], and (2) Mössbauer spectroscopy, which indicates that all three iron-sulfur centers (F_A, F_B and F_X) are [4Fe-4S] clusters [5,6]. Recently, however, EXAFS spectra of the Photosystem I iron were best modeled with a mixture of both [2Fe-2S] and [4Fe-4S] clusters [27]. This apparent discrepancy prompted us to use the core extrusion technique to investigate the type of iron-sulfur clusters in Photosystem I.

Identification of extruded iron-sulfur clusters is complicated by the large amount of chlorophyll (approx. 5 Chl/Fe) in the Photosystem I particle and by the possibility that reaction center may contain more than one type of cluster. We therefore used the ^{19}F NMR method in which para-tri-

Abbreviations: PS I, Photosystem I; Chl, chlorophyll; HMPA, hexamethylphosphoramide; DMSO, dimethylsulfoxide; R_FSH, *p*-trifluoromethylbenzenethiol.

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fluoromethylbenzenethiol displaces the cysteine thiolate ligands [7]. The ^{19}F NMR resonance positions with 1,1,1-monofluorodichlorotoluene as the reference are [2Fe-2S], −3.7 ppm; [4Fe-4S], −6.4 ppm; and free thiol, −8.0 pm with negative shifts being upfield from the reference.

Photosystem I reaction center particles were prepared as described previously [8]; acid-labile sulfide was assayed colorimetrically [9]. The particles were concentrated to 3 mg/ml Chl and stored in 0.05 M Tris buffer (pH 8.5) containing 0.05% Triton X-100. A standard solution of $(Et_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}CF_3)_4]$ was prepared, and *p*-trifluoromethylbenzenethiol and 1,1-dichloro-1-monofluorotoluene were synthesized as reported in Ref. 7.

The HMPA-based extrusion was carried out under argon according to the following protocol. A 0.25 ml aliquot of Photosystem I particle (3 mg/ml Chl, 750 μM in Fe) was degassed and combined with 1.0 ml of HMPA and 20 μl of R_FSH ($R_FSH/Fe > 100$). After incubation for 1 h at room temperature, 0.75 ml of the extrusion mixture and 1 μl of the reference (1,1-dichloro-1-monofluorotoluene) were transferred to a 5 mm septum-capped NMR tube via a gas-tight syringe. This tube was placed in the probe of a Bruker WM-300 NMR spectrometer operating at 282.4 MHz for ^{19}F and cooled to −15°C to achieve slow exchange between free and bound thiol. The ^{19}F NMR spectrum was taken by accumulating a total of 48000 scans; 1 μl of synthetic [4Fe-4S] cluster standard solution was injected and another spectrum was obtained under identical conditions. Areas of the peaks were determined by planimetric integration.

Fig. 1 shows a ^{19}F NMR spectrum of the extruded iron-sulfur clusters from Photosystem I before (bottom) and after (top) the addition of the synthetic [4Fe-4S] cluster standard. The spectrum of extruded Photosystem I shows not only [4Fe-4S] clusters at −6.4 ppm, but also presence of [2Fe-2S] clusters at −3.7 ppm. From the peak areas in the bottom spectrum, the ratio of [4Fe-4S] to [2Fe-2S] is 2.4 : 1. Since both [2Fe-2S] and [4Fe-4S] clusters have four ^{18}F thiols as ligands, the ratio is a rough estimate of the relative amount of each species present. Thus, although the majority of Photosystem I is extruded as [4Fe-4S] cluster, a significant

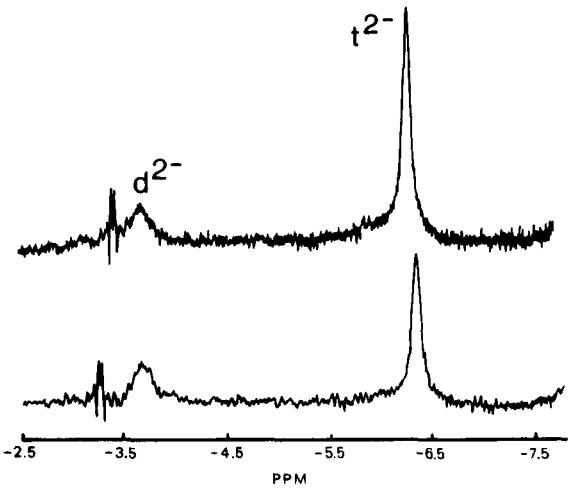

Fig. 1. ^{19}F NMR spectra of 4:1 v/v HMPA/H_2O (0.05 M Tris (pH 8.5)/0.05% Triton X-100) solutions of (bottom) Photosystem I protein at −15°C after completion of active site core extrusion reactions and (top) after addition of standards. The relatively sharp feature near −3.2 ppm is due to an instrumental artifact.

amount is extruded as [2Fe-2S] cluster. The ratio of [4Fe-4S] to [2Fe-2S] cluster varied somewhat from sample-to-sample; however, in no instance did we fail to find the presence of [2Fe-2S] clusters.

We did not, however, achieve quantitative removal of the available S^{2-} from the Photosystem I reaction center; the total sulfide extruded as [4Fe-4S] cluster varied between 39 and 89%. When the extrusion of Photosystem I was carried out in 66% HMPA instead of 80% HMPA, 40% of the total sulfide was extruded as [4Fe-4S] cluster and very little [2Fe-2S] cluster was found (data not shown). Slightly more [2Fe-2S] cluster was found if the sample was allowed to incubate overnight at room temperature. It should be noted that the iron-sulfur complement in *Azotobacter vinelandii* or *Clostridium pasteurianum* Fe-Mo Protein (nitrogenase) also could not be quantitatively extruded in 80% HMPA using *p*-trifluoromethylbenzenethiol as extruding agent; efficiencies ranged from 33 to 58% [7]. Similar to the case of nitrogenase, we consider it likely that the incomplete extrusion results from insufficient unfolding of the Photosystem I peptides in 80% HMPA.

Extrusions were also carried out in a medium containing 5% Triton X-100 and 3.6 M urea fol-

lowing a published protocol [10]. A sample of Photosystem I was degassed and diluted 1 : 3 (v/v) in 5% aqueous Triton-urea containing 50 mM thiol. After incubation for 1 h at room temperature, 0.75 ml was placed in an NMR tube accompanied by 1 μl of the chemical shift reference. The spectra of extruded Photosystem I showed the presence of [2Fe-2S] as well as [4Fe-4S] clusters (data not shown). These extrusions also were not quantitative; nevertheless, [2Fe-2S] clusters were present in every spectrum.

Thus, although the extrusion experiments in both 80% HMPA and 5% Triton-3.6 M urea failed to achieve quantitative removal of S^{2-} as $[Fe_nS_n(SR_F)_4]^{2-}$, the interesting result is that [2Fe-2S] clusters as well as [4Fe-4S] clusters appear in every extrusion mixture. The presence of [2Fe-2S] cluster is probably not due to the spontaneous conversion of $[Fe_4S_4(SR_F)_4]^{2-}$ to $[Fe_2S_2(SR_F)_4]^{2-}$, since this interconversion has not been observed in the 80% HMPA extrusion medium [7]. However, if a [4Fe-4S] cluster is oxidatively converted to a [3Fe-*X*S] cluster ($X = 3$ or 4), the core extrusion technique can show a [2Fe-2S] cluster [11,12]. The most likely source of oxidatively damaged [4Fe-4S] cluster is F_A and/or F_B [13]. However, chemical analysis shows a 1 : 1 ratio of S^{2-}/Fe^{3+} in freshly prepared Photosystem I reaction centers [13] and ESR integrations show a 1 : 1 ratio of P-700$^+$ with F_A^- [14,15] and F_B^- [15]. It is therefore unlikely that enough F_A or F_B would be damaged to correspond to the relatively large amount of [2Fe-2S] cluster found in Fig. 1. The only possible source of oxidant would be oxygen, and the excess thiol should be capable of reducing any oxygen present under these anaerobic conditions. The possibility that functional [3Fe-*X*S] clusters exist in Photosystem I can also be discounted: the redox potentials of F_A, F_B and F_X are too low to correspond to a usual [3Fe-*X*S] cluster [16] and there is no evidence in Photosystem I for a symmetrical ESR signal centered at $g = 2.01$ characteristic of an oxidized [3Fe-*X*S] cluster [17]. We conclude that the extruded [2Fe-2S] clusters represent inherent protein clusters in Photosystem I.

A functional Photosystem I reaction center contains 12 ± 2 mol of non-heme iron and labile sulfide per mol P-700 [3,18]. As indicated earlier, the ESR and Mössbauer data provide evidence for the identity of F_A and F_B as [4Fe-4S] clusters [4,5]. No ESR data are available on the identity of F_X [see Ref. 4]; however, given the problems encountered in Fig. 1 with 80% HMPA, it is possible that insufficient unfolding of the F_X-containing peptide took place in 80% DMSO to render the ESR experiment possible. Also, the Mössbauer data of F_X are compromised by a low signal-to-noise ratio and by the lack of quantitative reduction of F_X during the analysis [6]. Therefore, if F_A and F_B are considered [4Fe-4S] clusters, F_X could consist of either one or two [2Fe-2S] clusters or one [4Fe-4S] cluster. The best choice among all the possibilities is that the [4Fe-4S] clusters represent F_A and F_B and that the [2Fe-2S] clusters represent F_X.

This conclusion is supported by studies of the differential sensitivity of F_X relative to F_A and F_B. F_B suffers greatest oxidative denaturation, while F_X survives with the least amount of damage following treatment with urea-ferricyanide solution [13]. The clusters corresponding to F_A and F_B are known to be inactivated by short duration exposure to LDS, whereas F_X is not [19]. Indeed, LDS treatment of the Photosystem I reaction center removes the peptides corresponding to F_A and F_B without affecting the integrity of F_X (Golbeck, J.H., Parrett, K. and McDermott, A.E., unpublished results). Unfortunately, the relative instability of F_X in the presence of detergents precluded an extrusion of F_X on this new, minimal polypeptide particle.

F_X is known to be located on the 64-kDa, integral membrane protein that contains P-700 (Ref. 19; see also Golbeck, J.H., Parrett, K. and McDermott, A.E., unpublished data). F_A and F_B, on the other hand, may be located on a surface-exposed 8-kDa peptide in Photosystem I [20]. Analysis of this protein not only shows 7 ± 1 cysteine residues, but an amino acid content that is similar to that of bacterial ferredoxins that contain two [4Fe-4S] clusters. In these proteins, there is insufficient peptide to enclose completely the iron-sulfur clusters, rendering them partly exposed to the external environment [21]. It is therefore probable that enough unfolding occurs in 80% HMPA to cause large-scale extrusion of F_A and F_B. F_X, on the other hand, is probably more sequestered than either F_A or F_B, and the extrusion of these cluster(s)

may be relatively difficult. It might also be expected that unfolding of a high molecular weight, highly hydrophobic protein such as the 64-kDa peptide might be less efficient than that of a small, exposed 8-kDa peptide. This may explain why a relatively large amount of [4Fe-4S] clusters were seen in every extrusion experiment, while the most [2F-2S] cluster was seen in the sample containing 80% HMPA and the least in the 66% HMPA sample.

With this new information available on the identity of the bound iron-sulfur centers, we can begin to construct a plausible model for the Photosystem I reaction center. The nucleotide sequences for two light-inducible apoproteins of Photosystem I are known [22]. The PS I-A1 and PS I-A2 genes in maize chloroplasts are 45% homologous and encode polypeptides of 83.2 and 82.5 kDa. It has recently been shown that the upper approx. 64 kDa protein on SDS-polyacrylamide gel electrophoresis corresponds to the PS I-A1 gene product and the lower protein to the PS I-A2 gene product [22,23]. Both gene products are therefore present as integral components of the Photosystem I reaction center. The nucleotide sequence shows the presence of four and two cysteine residues, respectively, on the peptides PS I-A1 and PS I-A2. According to the hydropathy plot [22], the cysteines at positions 160 and 444 of PS I-A1 are located in 2 of the 11 membrane-buried helices. The cysteines at positions 575 and 584 of PS I-A1 are conserved in positions 560 and 569 of PS I-A2 and are located in extramembrane regions. Since four cysteine residues are assumed to bind both [2Fe-2S] and [4Fe-4S] clusters, it is possible that PS I-A1 by itself could hold one iron-sulfur cluster or that PS I-A1 and PS I-A2 share F_X as an interpolypeptide iron-sulfur cluster [19]. While the latter model meets the minimum requirement for two different high molecular weight peptides, a tetrameric protein structure composed of 64-kDa peptides best satisfies the stoichiometric data indicating four high-molecular weight peptides in *Synechococcus* Photosystem I [18]. The molecular weight of this complex would be about 350–400 kDa, which is compatible with the weight of 300–400 kDa determined by electron microscopy of the Photosystem I complex from *Synechococcus* [24].

If the higher plant reaction center is also a tetramer, two different structures might be delineated. Assuming cysteine serves as ligand to all of the iron atoms, it is possible that (1) a [2Fe-2S] cluster would be contained entirely within PS I-A1, or (2) a [2Fe-2S] cluster would bridge PS I-A1 and PS I-A2. The latter model, shown in Fig. 2, is attractive because it suggests a role for the homologous cysteines in PS I-A2, and because it provides a facile mechanism for the deterioration of F_X without destruction of P-700 or A_0. In either case, we suggest that in the complete reaction center, F_X is a pair of [2Fe-2S] clusters in which each [2Fe-2S] cluster in contributed by a two peptide subunit of Photosystem I. The pair of [2Fe-2S] clusters would form when two of these subunits associate to form the tetrameric Photosystem I reaction center. Since F_A and F_B are probably [4Fe-4S] clusters, the presence of 3.8 mol zero-valence sulfur [19] and 4.3 mol non-heme iron [28] on the isolated high-molecular-weight protein are compatible with the identification of F_X as a pair of [2Fe-2S] clusters. Two independent [2Fe-2S] clusters would require a total of eight cysteine residues as ligands to the iron atoms. A tetrameric arrangement of two PS I-A1 and two PS I-A2 peptides could provide sufficient cy-

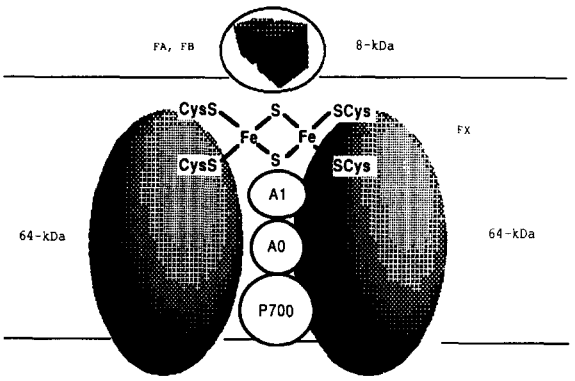

Fig. 2. Cross-sectional model of the Photosystem I reaction center based on stoichiometric data presented in the text. The complete reaction center would consist of four approx. 64-kDa peptides which could be a symmetrical pair of the approx. 130 kDa subunits shown here. The two approx. 130 kDa subunits would be positioned so that the pair of [2Fe-2S] clusters would be in magnetic interaction, thereby constituting F_X. The intact reaction center core would have a molecular weight of approx. 250 kDa.

steines to hold two iron-sulfur clusters; a dimeric arrangement of one PS I-A1 and one PS I-A2 peptide would not.

An interaction of two iron-sulfur clusters has been postulated to explain some of the unusual properties exhibited by F_X [27]. For example, the low g_{av} value of 1.91, the extremely broad ESR resonances, and the low redox potential are highly unusual for a [4Fe-4S] cluster [see Ref. 25]. Moreover, the ESR microwave power saturation characteristics have indicated that F_X cannot be a typical [2Fe-2S] or [4Fe-4S] cluster [26]. The model of F_X suggested here, while speculative, provides for a novel structure through the interaction of two iron-sulfur clusters while preserving the fundamental stoichiometry of the Photosystem I components.

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References

1 Evans, M.C.W. (1982) in Iron-Sulfur Proteins (Spiro, T.G., ed.), pp. 249–284, John Wiley & Sons, New York
2 Rutherford, A.W. and Heathcote, P. (1985) Photosyn. Res. 6, 295–316
3 Golbeck, J.H. (1987) J. Membrane Sci., in the press
4 Cammack, R. and Evans, M.C.W. (1975) Biochim. Biophys. Res. Commun. 67, 544–549.
5 Evans, E.H., Rush, J.D., Johnson, C.E. and Evans, M.C.W. (1979) Biochem. J. 182. 861–865
6 Evans, E.H., Dickson, D.P.E., Johnson, C.E., Rush, J.D. and Evans, M.C.W. (1981) Eur. J. Biochem. 118, 81–84
7 Wong, G.B., Kurtz, D.M., Holm, R.H., Mortenson, L.E. and Upchurch, R.G. (1979) J. Am. Chem. Soc. 101, 3078–3090
8 Golbeck, J.H. (1980) Methods Enzymol. 69, 129–141
9 Golbeck, J.H. and San Pietro, A. (1976) Anal. Biochem. 73, 539–542
10 Kurtz, D.M. (1983) Inorg. Chim. Acta 80, 75–76
11 Kurtz, D.M., Holm, R.H., Ruzicka, R.J., Beinert, H., Coles, C.J. and Singer, T.P. (1979) J. Biol. Chem. 254, 4967–4969
12 Beinert, H., Emptage, M.H., Dreyer, J.-L., Scott, R.A., Hahn, J.E., Hodgson, K.O. and Thomson, A.J. (1983) Proc. Natl. Acad. Sci. USA, 80, 393–396.
13 Golbeck, J.H. and Warden, J.T. (1982) Biochim. Biophys. Acta 681, 77–84
14 Bearden, A.J. and Malkin, R. (1972) Biochim. Biophys. Acta 283, 456–468
15 Heathcote, P., Williams-Smith, D.-L. and Evans, M.C.W. (1978) Biochem. J. 170, 373–378
16 Emptage, M., Dreyer, J.-L., Kennedy, M.C. and Beinert, H. (1983) J. Biol. Chem., 11106–11111
17 Johnson, M.K., Bennett, D.E., Morningstar, J.E., Adams, M.W.W. and Mortenson, L.E. (1985) J. Biol. Chem. 260, 5456–5463
18 Lundell, D.J., Glazer, A., Melis, A. and Malkin, R. (1985) J. Biol. Chem. 260, 646–654
19 Golbeck, J.H. and Cornelius, J.T. (1986) Biochim. Biophys. Acta 849, 16–24
20 Lagoutte, B., Setif, P. and Duranton, J. (1984) FEBS Lett. 174, 24–29
21 Jensen, L.H., Sieker, L.C., Watenpaugh, K.D., Adman, E.T. and Herriott, J.R, (1973) Biochem. Soc. Trans. 1, 27–29
22 Fish, L.E., Kuck, U. and Bogorad, L. (1985) J. Biol. Chem. 260, 1413–1421
23 Fish, L.E. and Bogorad, L. (1986) J. Biol. Chem. 261, 8134–8139
24 Williams, R.C., Glazer, A.N. and Lundell, D.J. (1983) Proc. Natl. Acad. Sci. 80, 5923–5926
25 Warden, J.T. and Golbeck, J.H. (1986) Biochim. Biophys. Acta 849, 25–31
26 Rupp, H., Rao, K.K., Hall, D.O. and Cammack, R. (1978) Biochim. Biophys. Acta 537, 255–269
27 McDermott, A.E., Yachandra, V.K., Guiles, R.D., Britt, R.D., Dexheimer, S.L., Sauer, K. and Klein, M.P (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. I, pp. 565–568, Martinus Nijhoff, Dordrecht
28 Høj, P. and Møller, B.L. (1986) J. Biol. Chem., 261, 14292–14300