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ON THE SITE OF FUNCTION OF THE RIESKE IRON-SULFUR CENTER IN THE CHLOROPLAST ELECTRON TRANSPORT CHAIN

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Summary

A photosynthetic mutant (strain 1073) of *Lemna perpusilla* was previously shown to have a block in the electron transport chain between plastoquinone and cytochrome *f* ((1976) Plant Physiol. 57, 577—579). Electron paramagnetic resonance analysis of chloroplasts from this mutant indicates that the $g = 1.89$ signal of a reduced iron-sulfur center (the 'Rieske' iron-sulfur center) is absent. The absence of this signal indicates the Rieske center is either absent from or defective in the mutant, and this result is consistent with this iron-sulfur center functioning between plastoquinone and cytochrome *f* in the electron transport chain of chloroplasts.

A new iron-sulfur center with a relatively high oxidation-reduction midpoint potential ($E_m = +290$ mV) was recently detected in chloroplasts [1]. This center has properties similar to those of an iron-sulfur center first discovered in mitochondria [2,3] and subsequently found in chromatophores from several photosynthetic bacteria [4—6]. The site of function of this center, known as the "Rieske" iron-sulfur center, in the chloroplast electron transfer chain is not yet known.

At approximately the same time as the Rieske center was being characterized in spinach chloroplasts, Shahak, Posner and Avron [7], in studies with a photosynthetic mutant of *Lemna perpusilla* (duckweed), found that this strain had a block in the photosynthetic electron transport chain between plastoquinone and cytochrome *f*. These workers knew of no electron transfer components in this region and therefore could not relate the block to any missing or defective component. In this communication, we report an analysis

of chloroplasts from the wild-type *Lemna* and mutant strain 1073 which indicates the mutant lacks the $g = 1.89$ electron paramagnetic resonance (EPR) signal of the Rieske iron-sulfur center. This finding is consistent with the Rieske center functioning between plastoquinone and cytochrome *f* in the chloroplast electron transfer chain.

The growth of wild-type *Lemna perpusilla* and mutant strain 1073 and the preparation of chloroplasts were as previously described [7]. Chloroplast samples were resuspended in washing medium containing 5% DMSO. Samples were frozen in solid CO_2 and shipped to Berkeley from Binghamton. The samples were then thawed and concentrated to a chlorophyll concentration of 2 mg/ml by centrifugation and resuspension in buffer. Analysis of the Rieske iron-sulfur center was done by EPR spectroscopy at 15 K. Chloroplast samples (0.3 ml) were incubated in the dark with 0.25 mM durohydroquinone at 4C for 2 min to reduce the Rieske center. The samples were then frozen to 77 K and EPR analysis was carried out as previously described [1,8,9].

The Rieske iron-sulfur center has a principal *g* value of 1.89 and a linewidth of approximately 40 G in the reduced state [1]. As shown in Fig. 1, the EPR signal from this center is observed in chloroplasts from wild-type *Lemna* after reduction with durohydroquinone. The EPR properties and temperature dependence of the signal in *Lemna* chloroplasts are similar to those in spinach chloroplasts. Fig. 1 also shows that the Rieske center signal was not detected in mutant chloroplasts reduced with durohydroquinone. Based on the limits of detection of this center by the EPR procedure, we estimate that the mutant chloroplasts contain no more than 10% of the amount of the Rieske center found in wild-type chloroplasts.

Previous results [7] on electron transfer reactions and electron carriers in the *Lemna* mutant led to the conclusion that the mutant lacked a functional carrier between plastoquinone and cytochrome *f*. Our results indicate this

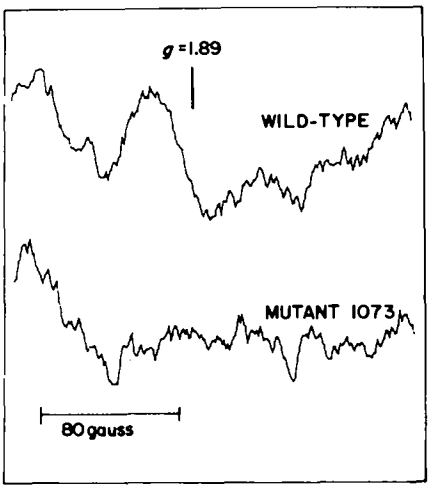

Fig. 1. The $g = 1.89$ EPR signal of the Rieske iron-sulfur center in *Lemna perpusilla* chloroplasts. Chloroplasts, at a chlorophyll concentration of 2 mg per ml, from the wild-type or mutant plants were reduced with 0.25 mM durohydroquinone prior to freezing at 77 K. EPR conditions: field setting, 3500 ± 250 G; microwave power, 10 mW; modulation amplitude, 10 G; temperature, 15 K.

carrier is most likely the Rieske iron-sulfur center. This site of function is consistent with the reported oxidation-reduction midpoint potentials of plastoquinone (+80 mV, pH 7.0, [10]), the Rieske iron-sulfur center (+290 mV, [1]), and cytochrome *f* (about +350 mV, [11—13]).

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References

1 Malkin, R. and Aparicio, P.J. (1975) Biochem. Biophys. Res. Commun. 63, 1157—1160
2 Rieske, J.S., MacLennan, D.H. and Coleman, R. (1964) Biochem. Biophys. Res. Commun. 15, 338—344
3 Rieske, J.S., Hansen, R.E. and Zaugg, W.S. (1964) J. Biol. Chem. 289, 3017—3022
4 Evans, M.C.W., Lord, A.V. and Reeves, S.G. (1974) Biochem. J. 138, 177—183
5 Prince, R.C., Leigh, Jr., J.S. and Dutton, P.L. (1974) Biochem. Soc. Trans. 2, 950—953
6 Prince, R.C., Lindsay, J.G. and Dutton, P.L. (1975) FEBS Lett. 51, 108—111
7 Shahak, Y., Posner, H.B. and Avron, M. (1976) Plant Physiol. 57, 577—579
8 Bearden, A.J. and Malkin, R. (1972) Biochim. Biophys. Acta 283, 456—468
9 Malkin, R. and Bearden, A.J. (1973) Biochim. Biophys. Acta 292, 169—185
10 Okayama, S. (1976) Biochim. Biophys. Acta 440, 331—336
11 Davenport, H.E. and Hill, R. (1952) Proc. R. Soc. Lond. Ser. B 139, 327—345
12 Malkin, R., Knaff, D.B. and Bearden, A.J. (1973) Biochim. Biophys. Acta 305, 675—678
13 Fan, H. and Cramer, W.A. (1970) Biochim. Biophys. Acta 216, 200—207