Short Communication

Restoration of High-Potential Cytochrome *b*-559 in Salt-Washed Photosystem II-Enriched Membranes as Revealed by EPR*

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Bergström, J. and Franzén, L.-G., 1987. Restoration of High-Potential Cytochrome *b*-559 in Salt-Washed Photosystem II-Enriched Membranes as Revealed by EPR. – Acta Chem. Scand., Ser. B 41: 126–128.

Photosystem II-enriched membranes contain cytochrome *b*-559, but the role of this component is still obscure. The cytochrome occurs in two forms, high-potential (HP, hydroquinone-reducible) and low-potential (LP, benzoquinone-oxidizable) (see Ref. 1 for a review). The high-potential form is labile and is converted to the low-potential form by many of the treatments that also cause a loss of O_2-evolution capacity.[2,3]

Washing of photosystem II membranes with high concentrations (1 M) of NaCl releases two extrinsic polypeptides of apparent molecular weights 16 and 24 kDa from the membrane with attendant partial inhibition of oxygen evolution. The extent of the inhibition varies, and ionic conditions in the assay, especially the Ca^{2+} and Cl^- concentrations, seem to be critical. Reactivation of oxygen evolution can be obtained either by rebinding of the 24 kDa polypeptide or by addition of millimolar concentrations of Ca^{2+} (see Ref. 4 for a review).

EPR has an advantage over optical methods in studies of the two forms of cytochrome *b*-559. The optical spectra of the high- and low-potential forms are identical, whereas the EPR spectra are different: HP, $g_z = 3.08$; LP, $g_z = 2.94$.[5]

In this work it is shown that the removal of the 16 and 24 kDa polypeptides induces total conversion of HP cytochrome *b*-559 to the low-potential form. Readdition of these polypeptides restores at least 50–70 % HP cytochrome *b*-559.

Materials and methods

Oxygen-evolving photosystem II-enriched membranes were prepared from spinach as in Ref. 6, with some modifications.[7,8] NaCl- and Tris-washing of the membranes were performed as in Ref. 8.

In order to keep HP cytochrome *b*-559 reduced and LP cytochrome *b*-559 oxidized in the reconstitution experiment, 4 mM of a hydroquinone/benzoquinone (1:1) redox buffer was added. For the low-temperature photooxidation experiments, 4 mM of sodium ascorbate was added to initially reduce both HP and LP cytochrome *b*-559. EPR measurements were performed as described in Ref. 5.

Results

In untreated photosystem II-enriched membranes about 30 % of the cytochrome *b*-559 is in its low-potential form (see Fig. 1; note that HP cytochrome *b*-559 is reduced and that only LP cyrochrome *b*-559 is detected in the EPR spectrum). Upon Tris-washing (not shown, see Ref. 5) and NaCl-washing, all of the HP cytochrome *b*-559 is converted to the low-potential form. This is observed both in the presence and absence of

*Communication at the 1st Swedish Photosynthesis Meeting in Stockholm, February 20–21, 1986.

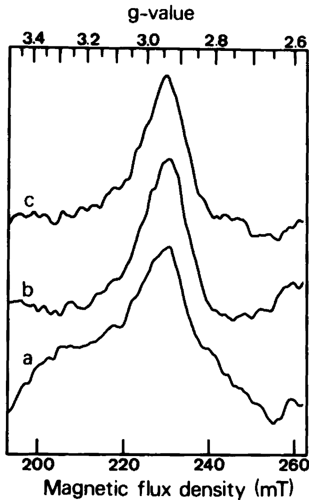

Fig. 1. Effects of salt-washing and readdition of the 16 and 24 kDa polypeptides on the EPR signal of LP cytochrome *b*-559 in photosystem II membranes (see also Table 1). All spectra are normalized with respect to the chlorophyll concentration. In order to keep LP cytochrome *b*-559 oxidized (EPR-detectable) and HP cytochrome *b*-559 reduced (EPR-invisible) a 4 mM hydroquinone/benzoquinone redox buffer was added. Spectrum (a) shows untreated membranes, (b) salt-washed membranes and (c) salt-washed membranes with 16 and 24 kDa polypeptides re-added with 10 mM $CaCl_2$ present. EPR conditions were: Temp. 15 K, microwave frequency 9.42 GHz, microwave power 20 mW, and modulation amplitude 3.2 mT.

Table 1. Effects of reconstitution on salt-washed photosystem II membranes.

	O_2-evolution[a] /%	Total LP cyt *b*-559 /%	NaCl-induced LP cyt *b*-559 /%
Control	100	29	0
Tris-washed	0	100	–
NaCl-washed	43	100	100
NaCl-washed + Ca^{2+} [b]	67	95	93
NaCl-washed + "16 and 24"[c]	89	60	44
NaCl-washed + Ca^{2+} + "16 and 24"	96	53	34

[a]The control activity was 656 μmol O_2 (mg chlorophyll)$^{-1}$ h^{-1}. [b]$[Ca^{2+}]$ = 10 mm. [c]Three-fold excess of 16 and 24 kDa polypeptides.

the hydroquinone/benzoquinone redox buffer. Tris-washing destroys all O_2-evolution activity, while NaCl-washing leaves about half of the activity intact. Addition of 10 mM $CaCl_2$ to the NaCl-washed photosystem II membranes does not change the amount of LP cytochrome *b*-559 significantly, but the O_2-evolution is enhanced. After readdition of the 16 and 24 kDa polypeptides, 40 % of the total and 56 % of the "NaCl-wash"-induced LP cytochrome *b*-559 is reconverted to the high-potential form. If Ca^{2+} is added together with the polypeptides the restoration of HP cytochrome *b*-559 is even better (see Table 1), and the O_2-evolution activity is then almost equal to the control value.

Ascorbate-reduced cytochrome *b*-559 can be photooxidized at cryogenic temperature.[5] Fig. 2 shows the EPR spectra of photooxidized cytochrome *b*-559 of untreated and Tris-washed photosystem II-enriched membranes. Note that Tris-washed membranes (where all cytochrome *b*-559 is in the low-potential form) give the same type of EPR spectrum (Fig. 2b) upon photooxidation as the untreated membranes. Thus, when photooxidized, both low- and high-potential cytochrome *b*-559 give the same EPR signal. The *g*-value is the same as that for chemically oxidized HP cytochrome *b*-559, viz. $g_z = 3.08$.[5]

Discussion

The HP cytochrome *b*-559 is converted into the low-potential form by removal of the 16 and 24 kDa polypeptides. Although manganese is released from NaCl-washed membranes in the presence of reductants such as hydroquinone,[9] the conversion of HP cytochrome *b*-559 to the low-potential form is not caused by the removal of the manganese in our experiment. This is shown by the fact that the conversion is complete also in the absence of hydroquinone.

Readdition of the 16 and 24 kDa polypeptides

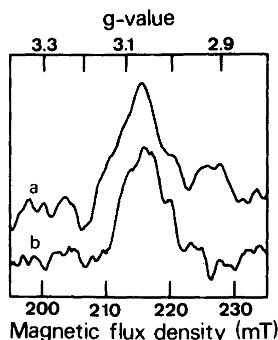

Fig. 2. Light–minus–dark EPR spectra of low-temperature photooxidized cytochrome *b*-559 in ascorbate-reduced photosystem II-enriched membranes. (a) Untreated membranes. (b) Tris-washed membranes. EPR conditions as for Fig. 1.

restores the HP cytochrome *b*-559. It has been proposed[10,11] that a hydrophobic environment is required to stabilize the high-potential form. Thus, rebinding of the polypeptides may serve to create the necessary hydrophobicity in the neighbourhood of the cytochrome. A close association between the 24 kDa polypeptide and cytochrome *b*-559 has also been observed in immunological studies.[12]

The interconvertibility of the high- and low-potential forms could be important for a role of cytochrome *b*-559 in proton binding during the catalytic cycle of water-splitting.[13,14] However, there appears not to be any direct correlation between O_2-evolution and the amount of HP cytochrome *b*-559 present (Table 1). As shown in Fig. 2, photooxidation of LP cytochrome *b*-559 at low temperature results in an EPR spectrum typical of HP cytochrome *b*-559. The correlation between high- and low-potential forms under static and flow conditions may therefore be different.

Another possible function for cytochrome *b*-559 could be in cyclic electron transport around photosystem II coupled to a vectorial proton transport across the thylakoid membrane. Cyclic electron transport with cytochrome *b*-559 involved has been observed under some conditions.[15] The orientation of the heme planes of the cytochrome, which are perpendicular to the membrane plane,[5] is consistent with such a role. The involvement of *b*-cytochromes in proton-translocation is reviewed in Ref. 16.

Acknowledgement. This work was supported by the Swedish Natural Science Research Council.

References

1. Bendall, D. S. *Biochim. Biophys. Acta 683* (1982) 119.
2. Larsson, C., Jansson, C., Ljungberg, U., Åkerlund, H. E. and Andersson, B. In: Sybesma, C., Ed., *Advances in Photosynthesis Research,* Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands 1984, Vol. I, p. 363.
3. Briantais, J. M., Vernotte, C., Miyao, M., Murata, N. and Picaud, M. *Biochim. Biophys. Acta 808* (1985) 348.
4. Ghanotakis, D. M. and Yocum, C. F. *Photosynth. Res. 7* (1985) 97.
5. Bergström, J. and Vänngård, T. *Biochim. Biophys. Acta 682* (1982) 452.
6. Berthold, D. A., Babcock, G. T. and Yocum, C. F. *FEBS Lett. 134* (1981) 231.
7. Ford, R. C. and Evans, M. C. W. *FEBS Lett. 160* (1983) 159.
8. Franzén, L.-G., Hansson, Ö and Andréasson, L.-E. *Biochim. Biophys. Acta 808* (1985) 171.
9. Ghanotakis, D. F., Topper, J. N. and Yocum, C. F. *Biochim. Biophys. Acta 767* (1984) 524.
10. Matsuda, H. and Butler, W. *Biochim. Biophys. Acta 724* (1983) 123.
11. Matsuda, H. and Butler, W. *Biochim. Biophys. Acta 725* (1983) 320.
12. Ljungberg, U., Jansson, C., Larsson, C., Åkerlund, H. E. and Andersson, B. In: Sybesma, D., Ed., *Advances in Photosynthesis Research,* Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands 1984, Vol. I, p. 367.
13. Butler, W. L. *FEBS Lett. 95* (1978) 19.
14. Webber, A. N., Spencer, L., Sawyer, D. T. and Heath, R. L. *FEBS Lett. 189* (1985) 258.
15. Heber, U., Kirk, M. R. and Boardman, N. K. *Biochim. Biophys. Acta 546* (1979) 292.
16. Wikström, M. and Saraste, M. In: Ernster, L., Ed., *Bioenergetics,* Elsevier, Amsterdam 1984, p. 49.

Received September 26, 1986.