REDOX PROPERTIES OF THE DBMIB–RIESKE IRON–SULFUR COMPLEX IN SPINACH CHLOROPLAST MEMBRANES

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1. Introduction

The plastoquinone analogue, 2,5-dibromo-3-methyl-6-isopropylbenzoquinone (DBMIB), has been used extensively as an inhibitor in studies of photosynthetic electron transport [1,2]. Recent electron paramagnetic resonance (EPR) results have indicated that DBMIB interacts with a high-potential iron–sulfur center, the Rieske ion–sulfur center, in chloroplasts and other photosynthetic membranes [3,4]. This interaction is manifested by a shift in the EPR *g*-value of the reduced iron–sulfur center from 1.89 in the absence of DBMIB to 1.94 in the presence of the inhibitor. In order to characterize the nature of this interaction in greater detail, studies of the redox potential dependence of the EPR signal of the DBMIB-inhibited system have been undertaken. The results are consistent with the presence of a DBMIB–iron–sulfur center complex in which the midpoint potential of the Rieske iron–sulfur center has been significantly altered from that in the untreated membranes. The complex can undergo a series of stepwise oxidation–reduction reactions which appear to be related to the redox state of DBMIB. One of these involves the semiquinone of DBMIB anti-ferromagnetically coupled to the reduced iron–sulfur center.

2. Materials and methods

Chloroplast membranes, prepared from freshly picked greenhouse spinach, were isolated as in [5]. Redox titrations of chloroplast samples in the presence or absence of DBMIB were carried out under anaerobic conditions, as in [6], and were done in the dark to prevent photoaccumulation of reduced iron–sulfur center A of the photosystem I primary electron acceptor complex since this reduced carrier has a *g*-value of 1.94 [7] which is identical to that of the DBMIB complex and would therefore interfere in this region. Samples poised at desired redox potentials were stored in the dark at 77 K for subsequent EPR analysis. EPR spectra were recorded at 15 K in a modified JEOL X-band spectrometer operating with 100 kHz field modulation [8].

DBMIB, a gift from Dr A. Trebst, was dissolved in ethanol as a 50 mM stock solution. The volume added to the reaction mixture was such that the final ethanol concentration did not exceed 1%.

3. Results

Previous results have characterized the reduced chloroplast Rieske iron–sulfur center with an EPR *g*-value of 1.89 and a midpoint potential of +290 mV at pH 8.0 [9]. As shown in fig.1, a similar value was obtained in recent titrations (E_m = +310 mV, n = 1), and no other redox transitions were observed in the potential range from +200 to –300 mV. The linewidth of the g = 1.89 signal was unchanged over this potential range, in contrast to changes in linewidth reported for the mitochondrial Rieske center as additional low-potential component underwent reduction in yeast complex III [10]. Examination of the EPR spectra in the g = 1.94 region as well as in the g = 1.89 region gave no indication of the presence of any other bound iron–sulfur centers with midpoint potentials from +200 mV to –300 mV. This result does not support the proposal [11] that an additional iron–sulfur center exists which is functional in an electrogenic loop in photosynthetic systems.

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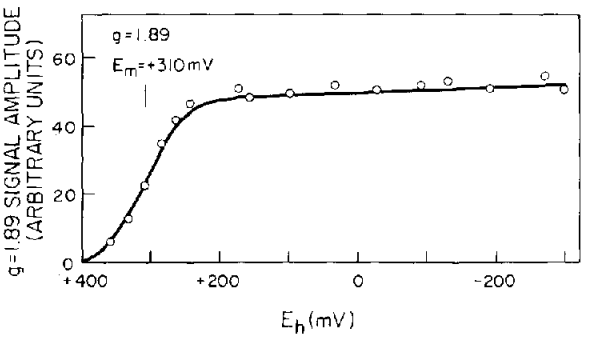

Fig.1. Oxidation–reduction titration of the Rieske iron–sulfur center in chloroplasts. The reaction mixture contained 0.1 M Tricine–KOH buffer (pH 8.0), 20 mM NaCl, chloroplasts at 3.3 mg chl/ml and the following redox mediators: tetramethylphenylenediamine (50 μM), 2,5-dimethylbenzoquinone (25 μM), 1,2-naphtoquinone (50 μM), phenazine methosulfate (50 μM), duroquinone (50 μM), anthraquinone disulfonate (50 μM), anthraquinone sulfonate (50 μM) and benzyl viologen (50 μM). The redox potential of the suspension was adjusted to ~−350 mV with sodium dithionite and on oxidative titration was done using 0.1 M potassium ferricyanide. EPR conditions: field setting, 3400 ± 250 G; microwave power, 5 mW; modulation amplitude, 10 G; temp. 12 K.

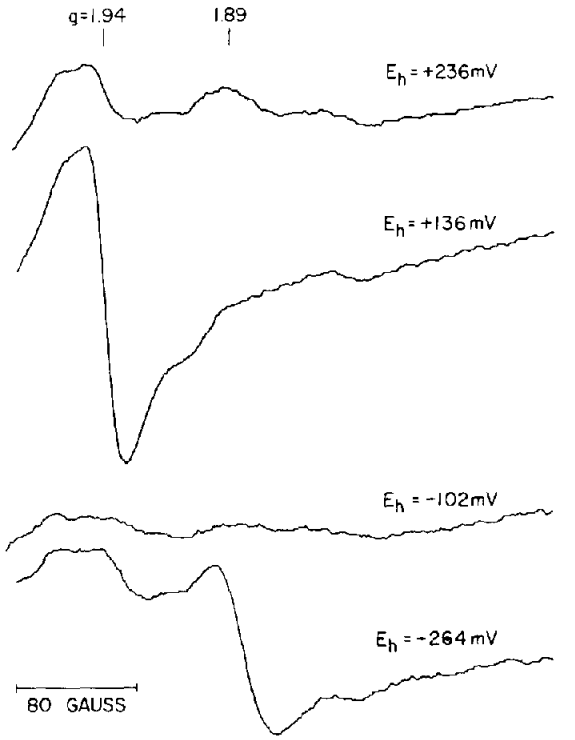

Fig.2. EPR spectra of the $g = 1.9$ region of chloroplasts in the presence of DBMIB as a function of redox potential. The reaction mixture was as in fig.1 except that DBMIB (100 μM) was present. Samples at the indicated potentials were removed for EPR analysis. EPR conditions were as in fig.1.

When DBMIB was added to the chloroplast suspension, a different redox titration behavior was observed. As shown in fig.2A, at a redox potential of +236 mV, where the Rieske center of the control sample was fully reduced, only a negligible $g = 1.89$ signal was present. Indeed, a small signal at $g = 1.94$ was observed. At a redox potential of +136 mV (fig.2B), the $g = 1.94$ signal had increased in amplitude, and still no $g = 1.89$ signal had appeared. At the more negative potential of −102 mV (fig.2C), the $g = 1.94$ signal had totally disappeared and no other signals were present. At the most negative potential in this series (−264 mV, fig.2D), a prominent $g = 1.89$ signal was now present with only a small residual signal present at $g = 1.94$. These 4 selected points suggest the appearance and subsequent disappearance of the $g = 1.94$ signal, followed by the appearance of a $g = 1.89$ signal at more negative potentials when DBMIB is present during the redox titration.

A complete titration curve in the presence of DBMIB is shown in fig.3. Two transitions, both showing $n = 1$ behavior, occurred at $g = 1.94$: this signal appeared with an $E_{m,\,8.0} = +180$ mV and then disappeared with an $E_{m,\,8.0} = +20$ mV. At lower potentials, a signal at $g = 1.89$ then appeared with an $E_{m,\,8.0} = -220$ mV ($n = 1$). All the transitions in this titration were reversible in that they exhibited the same E_m-values in both oxidative and reductive titrations.

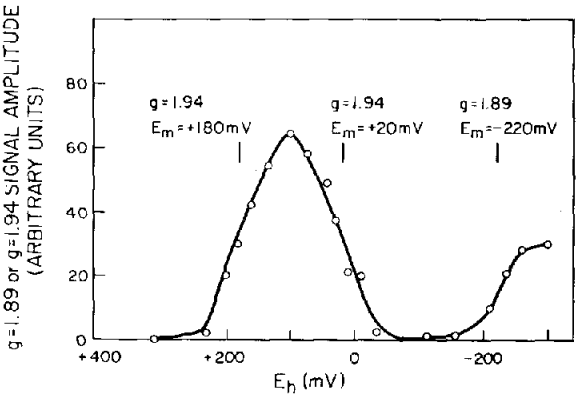

Fig.3. Oxidation–reduction titration of the chloroplast Rieske iron–sulfur center in the presence of DBMIB. Reaction mixture and EPR conditions were as in fig.1,2.

4. Discussion

The results of the present study of the redox properties of the Rieske iron–sulfur center in the presence of DBMIB indicate rather dramatic changes have occurred to this center. To explain the redox behavior shown in fig.3, the following scheme has been developed:

$$\mathrm{DBMIB_{ox}{-}FeS_{ox}} \xrightarrow{E_m = +180} \mathrm{DBMIB_{ox}{-}FeS_{red}} \xrightarrow{E_m = +20}$$

$$\mathrm{DBMIB^{\cdot}{-}FeS_{red}} \xrightarrow{E_m = -220} \mathrm{DBMIB_{red}{-}FeS_{red}}$$

According to this scheme, the oxidized inhibitor binds to the iron–sulfur center to form a quinone–iron–sulfur center complex with a g-value of 1.94 and an E_m = +180 mV. This is to be contrasted with a g-value of 1.89 and an E_m = +310 mV for the Rieske center in the absence of DBMIB. Subsequent redox reactions are then related to changes in the redox state of DBMIB. The +20 mV transition would correspond to the reduction of DBMIB to the semiquinone level; this semiquinone must interact strongly with the unpaired electron of the reduced iron–sulfur center. The complete disappearance of the g = 1.94 signal below +20 mV is consistent with an anti-ferromagnetic coupling between the two unpaired spins in the complex and would argue for a close physical association between the quinone and the iron–sulfur cluster of the Rieske center. If such a state exists, further reduction of DBMIB to the fully reduced state should eliminate this coupling and should result in a reappearance of the EPR signal of the reduced iron–sulfur center. The appearance of the g = 1.89 signal with an E_m = –220 mV is consistent with this prediction.

Two possibilities exist for the origin of the g = 1.89 signal at low potentials in the presence of DBMIB. The inhibitor–iron–sulfur center complex, as shown above, could still be present and the reduced DBMIB-reduced iron–sulfur center complex would then have a g-value at 1.89, which is the same as that of the reduced iron–sulfur center alone. Alternatively it is possible that the fully reduced inhibitor was displaced from the reduced iron–sulfur center, producing the signal at g = 1.89 from the reduced iron–sulfur center alone. Although our results do not allow a choice between these two cases, the results in [12] that DBMIB inhibits cyclic phosphorylation in dithionite-mediated systems and studies of DBMIB-inhibition of cytochrome photoreactions in the presence of reduced ferredoxin (R. Chain, unpublished) argue for the former explanation.

On the basis of the titration results of fig.3, it is possible to estimate an E_m for the quinone/quinol couple when DBMIB is complexed to the Rieske center, and a value of –100 mV is obtained. This value can be compared with $E_{m,\,8.0}$ = +60 mV reported for the corresponding couple of DBMIB in aqueous solution [13]. This decrease in E_m upon binding to the Rieske center reflects a tighter binding of the oxidized form of the inhibitor to the Rieske center as compared with the binding of reduced DBMIB. Furthermore, a semiquinone stability constant (K_s) of $\sim 10^4$ can be estimated for the DBMIB semiquinone complexed to the Rieske center (see [14,15] for a discussion of quinone stability constants). Although the K_s for DBMIB in solution is not known, K_s for durosemiquinone at alkaline pH has been reported to be ~1 [16] while bound ubisemiquinones in mitochondrial preparations have K_s values from $10–10^{-3}$ [17,18]. It would appear from a comparison of these values that a stabilization of several orders of magnitude for the DBMIB semiquinone has occurred through complex formation with the Rieske iron–sulfur center.

The results demonstrating the interaction of DBMIB with the Rieske center are noteworthy in relation to a general model currently emerging for electron transport in chloroplasts in which the oxidized Rieske center may function by interacting with plastohydroquinone and this electron transfer step produces plastosemiquinone and the reduced iron–sulfur center [11,19,20]. Similar proposals for the center's function in the mitochondrial and chromatophore electron-transport chains have been made on the basis of [21,22]. These results demonstrate for the first time that the Rieske center can interact with a semiquinone species of an inhibitory plastoquinone analogue. Further examination of the interaction of DBMIB and other quinones with this center may provide additional insights into the mechanism of electron transfer between quinones and the iron–sulfur center in energy-transducing membranes.

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