Interaction of stigmatellin and DNP-INT with the Rieske iron-sulfur center of the chloroplast cytochrome b_6-f complex

Richard Malkin

Division of Molecular Plant Biology, University of California, 313 Hilgard Hall, Berkeley, CA 94720, USA

Received 1 September 1986

Stigmatellin and DNP-INT are effective inhibitors of the catalytic activity of the plastoquinol-plastocyanin oxidoreductase complex (cytochrome b_6-f complex). Both inhibitors alter the EPR spectrum of the Rieske iron-sulfur center but do not produce band-shifts of cytochrome b-563. The midpoint redox potential of the Rieske center is unaffected by either inhibitor, although both alter the DBMIB-induced g-value shifts of the Rieske center. The results are considered in terms of binding domains for inhibitors in the cytochrome b_6-f complex.

Stigmatellin *DNP-INT* *Cytochrome* b_6-f *Rieske FeS protein* *DBMIB*

1. INTRODUCTION

Stigmatellin has recently been shown to be a potent inhibitor of the mitochondrial cytochrome *b-c* complex [1–3] and the chloroplast cytochrome b_6f complex [4]. In the former case, optical and EPR characterization of the interaction of the inhibitor with the complex has shown an effect on both cytochrome *b*-566 and the Rieske iron-sulfur center. The nature of the interaction with the chloroplast complex is as yet undefined.

Here, the effect of stigmatellin on the Rieske iron-sulfur center of the cytochrome b_6-f complex has been studied. The observed effects are similar to those of DNP-INT, a quinone analog found to be an effective inhibitor of the cytochrome b_6-f complex [5]. Although both these compounds affect the Rieske iron-sulfur center, their interaction is different from that of the halogenated quinone-type inhibitor, exemplified by DBMIB. The nature of the binding site of these different classes of inhibitors in the cytochrome b_6-f complex is also considered.

Abbreviations: DNP-INT, 2-iodo-2′,4′,4′-trinitro-3-methyl-6-isopropyl diphenyl ether; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DAD, diaminodurene; DCPIP, dichlorophenolindophenol

2. MATERIALS AND METHODS

The cytochrome b_6-f complex was prepared from spinach chloroplasts by the procedure of Hurt and Hauska [6], except that lipids were omitted from the final sucrose gradient step. The concentration of the complex was estimated from the ascorbate-reduced minus ferricyanide-oxidized difference spectrum of cytochrome *f* using an extinction coefficient of 18 $mM^{-1} \cdot cm^{-1}$ at 554 –540 nm.

EPR spectra were recorded at X-band in a Bruker 200ER tt spectrometer with 100 kHz modulation at approx. 15 K. Samples in 3 mm i.d. quartz tubes contained the cytochrome complex at a concentration of 10–12 μM cytochrome *f*. Redox titrations were performed at room temperature as previously described with ferricyanide as the oxidant and ascorbate as reductant [7].

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

DNP-INT and DBMIB were obtained from Professor A. Trebst, Bochum, and stigmatellin from Dr G. Höfle, Braunschweig. All inhibitors were dissolved in ethanol at a concentration of 5–10 mM and were added in microliter amounts.

3. RESULTS

The effect of DNP-INT and stigmatellin on the EPR lineshape of the Rieske iron-sulfur center in the cytochrome b_6-f complex was similar. The Rieske center is characterized by EPR g values of 2.03 and 1.90 in the reduced state (fig.1A), and the addition of either inhibitor at a 10–20-fold excess over the Rieske center produced a line narrowing as evidenced by an increase in signal intensity at g = 2.03 and 1.90 (fig.1B,C). Little or no g-value shift occurred. Lower concentrations of the inhibitors were less effective in producing these effects. In contrast to this behavior is the effect of DBMIB (fig. 1D), where marked g-value shifts are observed: the $g = 1.90$ line shifts to 1.94 and the $g = 2.03$ line shifts to 2.01. This effect has been previously reported and studied in some detail [8,9]. It has been found in this work that the DBMIB shifts are more pronounced when oxidized DBMIB and the oxidized Rieske center are allowed to interact prior to reduction with ascorbate. Under these conditions, a 2-fold excess of DBMIB over the Rieske center yields the observed g-value shifts.

In contrast to the results with the mitochondrial cytochrome b-c complex [3], stigmatellin did not induce any band shifts in the b-type cytochromes of the chloroplast complex (not shown). Other inhibitors, such as DNP-INT or myxothiazol, also failed to produce band shifts with the chloroplast complex.

Inhibitors which bind in the vicinity of the Rieske center based on changes in the center's EPR spectrum have also been found to alter the midpoint redox potential of the center [3,8,10,11]. As shown in fig.2, the Rieske center of the cytochrome b_6-f titrates with an E_m = 295 mV, and neither stigmatellin nor DNP-INT has signifi-

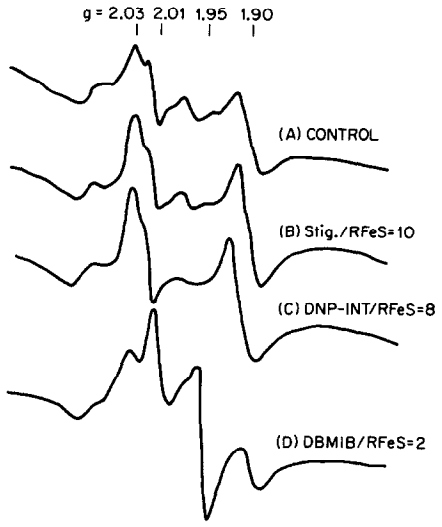

Fig.1. Effect of stigmatellin and DNP-INT on the EPR spectrum of the Rieske iron-sulfur center. The spinach cytochrome b_6-f complex (concentration, 13 μM cytochrome f) in 30 mM Tris-succinate buffer (pH 6.5) plus 1 mM EDTA, was incubated in DNP-INT or stigmatellin at the indicated concentration for 1 min at 25°C prior to the addition of 2 mM sodium ascorbate. (A) Control with no inhibitor, (B) DNP-INT/Rieske = 8, (C) stigmatellin/Rieske = 10, (D) DBMIB/Rieske = 2. EPR conditions: microwave power, 10 mW; modulation amplitude, 10 G; temperature, 15 K.

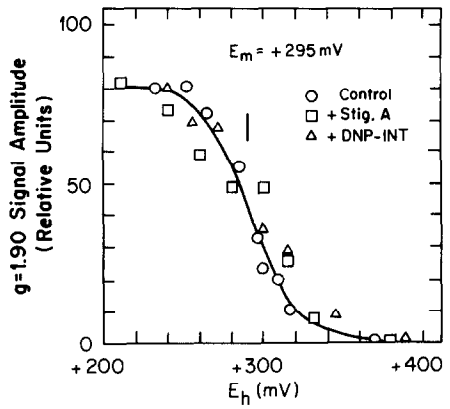

Fig.2. Redox titration of the Rieske center in the presence of DNP-INT or stigmatellin. The cytochrome b_6-f at a concentration of 13 μM cytochrome f was titrated at room temperature. The following redox mediators were present: 40 μM DAD and 50 μM DCPIP. Ferricyanide and ascorbate were used to adjust the potential of the reaction mixture and samples removed at indicated E_h values. EPR conditions were as in fig.1. The line through the points indicates a component with E_m = 295 mV ($n = 1$).

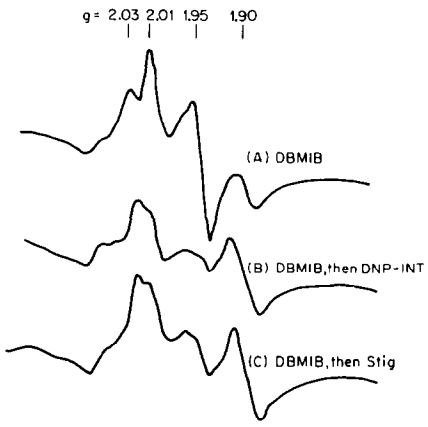

Fig.3. Effect of DNP-INT and stigmatellin on the DBMIB-induced EPR spectrum of the Rieske iron-sulfur center. The cytochrome b_6-f complex (concentration of cytochrome, 13 μM) was incubated with 26 μM DBMIB, followed by either DNP-INT (B) or stigmatellin (c) at a concentration of 130 μM (10-fold excess over the Rieske center) and then the sample reduced by the addition of 2 mM ascorbate. EPR conditions were as in fig.1.

cant effect on this E_m when added in 10–20-fold excess.

Stigmatellin and DNP-INT are capable of modifying the DBMIB-induced EPR signal of the Rieske center. As shown in fig.3B and C, the addition of either inhibitor after the addition of DBMIB causes a reappearance of the $g=1.94$ signal. In this experiment, an approx. 10-fold excess of stigmattelin or DNP-INT was used, and this concentration is comparable to that shown to be effective in fig.1.

4. DISCUSSION

Stigmatellin and DNP-INT act in a similar manner in relation to the Rieske iron-sulfur center of the cytochrome b_6-f complex. Both compounds have been shown to be effective inhibitors of quinol-plastocyanin oxidoreductase activity [4,5], and in this study, they both produce a line narrowing of the Rieske center EPR signal and alter the binding of DBMIB to this center. In contrast to the results with the mitochondrial cytochrome b-c complex, stigmatellin does not change the E_m of the Rieske center, nor does it cause a red shift in the spectrum of cytochrome b-563 [3]. DNP-INT shows similar properties in all respects.

The simplest interpretation of these results is in terms of two overlapping binding domains for inhibitors in the vicinity of the Rieske center. Halogenated quinones, i.e. DBMIB, bind most efficiently to a site near the iron-sulfur center of the Rieske protein site since they are effective in altering the properties of this center in almost stoichiometric amounts, and their effects on the Rieske center EPR signal and E_m are most dramatic. Other quinone analogs, such as DNP-INT and stigmatellin, must bind at an overlapping site, but this site must be more remote from the iron-sulfur cluster than the DBMIB site because effects on the EPR spectrum are less pronounced and no effect on the E_m of the center has been detected. Since the second group of inhibitors can affect the DBMIB binding, as evidenced by the shifts in g values seen in fig.3, there must be some close association between these two sites.

It is also clear from the results with stigmatellin that its mode of action in the chloroplast cytochrome complex differs dramatically from that in the mitochondrial cytochrome complex, i.e. no induced band shift of cytochrome b and no effect on the Rieske center midpoint redox potential. Thus, although functionally these two electron transfer complexes are similar and they contain the same set of conserved redox carriers, substantial differences also exist. Further comparative studies with inhibitors that interact with both complexes may provide additional insights into the significance of these differences.

ACKNOWLEDGEMENTS

This work was supported in part by a research grant from the National Institutes of Health and a fellowship from the Guggenheim Foundation. I would like to thank Professor Trebst and Dr. Höfle for the generous gifts of the inhibitors used and R.K. Chain for the preparation of the cytochrome complex.

REFERENCES

[1] Kunze, B., Kemmer, T., Höfle, G. and Reichenbach, H. (1984) J. Antibiot. 37, 454–461.
[2] Thierbach, G., Kunze, B., Reichenbach, H. and Höfle, G. (1984) Biochim. Biophys. Acta 765, 227–235.

[3] Von Jagow, G. and Ohnishi, T. (1985) FEBS Lett. 185, 311–315.
[4] Oettmeier, W., Godde, D., Kunze, B. and Höfle, G. Biochim. Biophys. Acta 807 (1986) 216–219.
[5] Hurt, E. and Hauska, G. (1981) Eur. J. Biochem. 117, 591–599.
[6] Hurt, E. and Hauska, G. (1982) J. Bioenerg. Biomembranes 14, 405–424.
[7] Knaff, D.B. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 244–252.
[8] Malkin, R. (1981) FEBS Lett. 131, 169–172.
[9] Malkin, R. (1981) Isr. J. Chem. 21, 301–305.
[10] Bowyer, J.R., Edwards, C.A., Ohnishi, T. and Trumpower, B.L., (1982) J. Biol. Chem. 257, 8321–8330.
[11] Matsuura, K., Bowyer, J.R., Ohnishi, T. and Dutton, P.L. (1983) J. Biol. Chem. 258, 1571–1579.