Reversal of Dibromothymoquinone Inhibition of Photosynthetic Electron Flow by Thiol Compounds

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Dibromothymoquinone inhibits photosynthetic NADP reduction by broken chloroplasts. The inhibition of electron flow and coupled ATP formation is effectively reversed by the addition of thiol compounds.

Dibromothymoquinone (DBMIB) is now a well established inhibitor of photosynthetic electron flow at the plastoquinone level [1, 2]. In particular DBMIB has permitted the study of the properties of photoreductions by just photosystem II in isolated thylakoid preparations from chloroplasts [2, 3]. A reversal of DBMIB inhibition of NADP reduction by the addition of exogenous plastoquinone [4] as well as a bypass of the inhibition site by TMPD [5] has been described. This communication reports on the effective reversal of DBMIB inhibition by thiol-reagents.

Results and Discussion

At a concentration of 10^{-6} M, DBMIB inhibits completely photosynthetic NADP reduction by isolated chloroplasts [1]. The addition of an excess of several compounds containing SH groups completely reverses NADP reduction as well as ATP formation coupled to it (Table I). As Table II indicates, a fivefold excess of dithiothreitol (DTT) is sufficient to overcome DBMIB inhibition, whereas a twofold excess is only partly effective.

Table I. Reversal of DBMIB inhibition by thiol compounds in photosynthetic NADP reduction by broken chloroplasts (pH 8.0).

Additions to 2×10^{-6} M DBMIB	Formation of NADPH [μmol/mg Chl·h]	ATP
control without DBMIB	129	126
— — —	6	0.1
5×10^{-3} M DTT	114	141
3×10^{-3} M cysteine	111	147
3×10^{-3} M glutathione	102	78
10^{-5} M mercaptoethanol	90	93

The sequence of addition of compounds is of importance. If DBMIB and DTT are preincubated for 5 min and then added to the chloroplast test system, no inhibition of NADP reduction is observed (Table II). If the chloroplasts and DBMIB are preincubated for 5 min, DTT, added later, is not able to overcome inhibition. A preincubation of DTT and chloroplasts is only partly effective in protecting the chloroplasts from DBMIB.

Table II. Dependence of the reversal of DBMIB inhibition on concentration and on the preincubation conditions in photosynthetic NADP reduction by broken chloroplasts.

	Rate of electron flow [μmol NADPH/mg Chl·h]
Control without DBMIB	105
2×10^{-6} M DBMIB	6
2×10^{-6} M DBMIB + 10^{-5} M DTT	93
5×10^{-6} M DBMIB + 10^{-5} M DTT	21
preincubation of DBMIB with DTT	135
preincubation of DBMIB with chloroplasts	6
preincubation of DTT with chloroplasts	42

The 1,4-addition of SH compounds to quinones is well known. The addition of thiols onto DBMIB would possibly lead to a splitting off of HBr and therefore to an inactive compound. The results reported might suggest that DBMIB acts also *in situ* by reacting with endogenous SH groups in the chloroplast, essential for photosynthetic electron flow and located in the region of the plastoquinone functional site. However, then one would expect that other even more effective SH scavengers do also inhibit photosynthetic electron flow in a DBMIB like manner, which has not been reported so far. Also the present properties of DBMIB inhibition of chloroplasts are not easily reconciled with the assumption of an attack of DBMIB on endogenous SH groups, but the possibility cannot be excluded.

The occasionally observed puzzling ineffectiveness of DBMIB on *in vivo* systems is very likely due to an inactivation of the inhibitor by endogenous thiols in the intact cells.

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