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SHORT COMMUNICATION

THE EFFECTS OF SOME SYNTHETIC QUINONES ON THE HILL REACTION IN SPINACH CHLOROPLASTS

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Abstract—The Hill reaction activity of spinach chloroplasts has been measured spectrophotometrically by photoreduction of 2,6-dichlorophenol indophenol. Tetrachlorobenzoquinone, 2,3-dichloronaphthoquinone, and 1,2-dihydroxyanthraquinone (alizarin) were found to inhibit the reaction rate, while 2,5-diphenylbenzoquinone and 1,8-dihydroxyanthraquinone were stimulatory.

INTRODUCTION

WITHIN recent years it has been found that plant chloroplasts contain a number of quinones. These include plastoquinone A (2,3-dimethyl,5-solanesylbenzoquinone),[1-3] plastoquinones B, C and D,[4,5] vitamin K_1 (2-methyl,3-phytyl,1,4-naphthoquinone),[6,7] α-, β- and γ-tocopherols and the corresponding tocopherylquinones,[8] and an as yet unidentified naphthoquinone.[9] It has been shown that plastoquinone A,[10-12] plastoquinones C and D,[13-14] and tocopherylquinones[13-14] will restore the photochemical activity of chloroplasts which have been extracted with organic solvents, and are thus essential components of the electron transport system of the chloroplast.

Redfearn and Friend[15] performed experiments with chloroplasts extracted with petroleum ether to which extraneous quinones were added, and tested for reactivation of Hill activity. A number of 1,4-benzoquinone derivatives not only restored Hill reaction activity, but even stimulated the activity of unextracted preparations. Both trimethyl- and 2-methoxy,6-propyl,1,4-benzoquinone were more active than plastoquinone itself. On the other hand, ubiquinone-24, phthiocol and several vitamin K_2 derivatives were inhibitory.

The present experiments are concerned with the effects of tetrachlorobenzoquinone,

[1] N. I. BISHOP, *Proc. Natl. Acad. Sci. U.S.* **45**, 1696 (1959).
[2] F. L. CRANE, *Plant Physiol.* **34**, 128 (1959).
[3] M. J. BECKER, J. A. GROSS and A. M. SHEFNER, *Biochim. et Biophys. Acta* **64**, 579 (1962).
[4] L. P. KEGEL, M. D. HENNINGER and F. L. CRANE, *Biochim. Biophys. Res. Commun.* **8**, 294 (1962).
[5] M. D. HENNINGER and F. L. CRANE, *Plant Physiol.* **39**, 598 (1964).
[6] L. P. KEGEL and F. L. CRANE, *Nature* **194**, 1282 (1962).
[7] H. K. LICHTENTHALER, *Planta* **57**, 731 (1962).
[8] R. A. DILLEY and F. L. CRANE, *Plant Physiol.* **38**, 452 (1963).
[9] M. MCKENNA, M. D. HENNINGER and F. L. CRANE, *Nature* **203**, 524 (1964).
[10] N. I. BISHOP, *Proc. Natl. Acad. Sci. U.S.* **45**, 1696 (1959).
[11] M. J. BECKER, J. A. GROSS and A. M. SHEFNER, *Biochim. et Biophys. Acta* **64**, 579 (1962).
[12] J. FRIEND and E. R. REDFEARN, *Phytochem.* **2**, 397 (1963).
[13] M. D. HENNINGER and F. L. CRANE, *Biochemistry* **2**, 1168 (1963).
[14] R. A. DILLEY and F. L. CRANE, *Plant Physiol.* **39**, 33 (1964).
[15] E. R. REDFEARN and J. FRIEND, *Biochem. J.* **84**, 34 (1962).

2,5-diphenylbenzoquinone, 2,3-dichloronaphthoquinone, 2-chloroanthraquinone, 1,2-dihydroxyanthraquinone (alizarin) and 1,8-dihydroxyanthraquinone on the Hill reaction activity of spinach chloroplasts, as measured spectrophotometrically using 2,6-dichlorophenol indophenol as the Hill oxidant.

RESULTS

A light intensity of 500 lux was used in all experiments. Rates of dye reduction of the order of 40–50 absorptivity units/min·mg chlorophyll were routinely obtained (Table 1). It may be seen that a considerable degree of inhibition was obtained with tetrachlorobenzoquinone, 2,3-dichloronaphthoquinone and 1,2-dihydroxyanthraquinone (alizarin). No significant differences from control rates were shown by 2-chloroanthraquinone, while increases in the rate were consistently obtained with 2,5-diphenylbenzoquinone and 1,8-dihydroxyanthraquinone.

Table 1. Effects of various quinones upon the Hill reaction activity of spinach chloroplasts

Compound	ΔA/min.mg chlorophyll		% Change
	Control rate	Experimental rate	
Tetrachlorobenzoquinone	27·2	17·2	−36·8
2,5-Diphenylbenzoquinone	47·2	54·7	−15·9
2,3-Dichloronaphthoquinone	32·7	19·0	−41·9
2-Chloroanthraquinone	50·1	52·1	− 4·0
1,2-Dihydroxyanthraquinone	44·1	25·0	−43·3
1,8-Dihydroxyanthraquinone	51·6	58·9	+14·2

DISCUSSION

While the maximal concentrations of the test quinones in suspension in the reaction mixture approximated $3{\cdot}3 \times 10^{-5}$, these compounds are so sparingly water soluble that the concentrations in solution were considerably lower. Complete inhibition of Hill reaction activity, such as may be obtained with *o*-phenanthroline for example, was not obtained in any instance, the extreme water insolubility of these compounds limiting their effectiveness. Both tetrachlorobenzoquinone and 2,3-dichloronaphthoquinone are commercial fungicides (trade names "Spergon" and "Phygon" respectively) and the present findings suggest that they might not be devoid of adverse effects upon higher plants.

The finding that the Hill reaction is stimulated by both 2,5-diphenylbenzoquinone and 1,8-dihydroxyanthraquinone adds further examples to the quinones earlier found to be stimulatory.[16] The present results provide a further demonstration of the essential nature of endogenous quinones in the electron transport system of the Hill reaction.

EXPERIMENTAL

Chloroplasts were prepared from fresh spinach by differential centrifugation in 0·5 M sucrose at 4°. The reactions were carried out in 25 ml Erlenmeyer flasks containing 8 ml of

[16] E. R. Redfearn and J. Friend, *Biochem. J.* **84**, 34 (1962).

the reaction mixture, consisting of 2 ml 2,6-dichlorophenolindophenol (0·2 mg/ml), 2 ml of the test quinone (0·1 mg/ml suspension), 0·2 ml of the chloroplast suspension, and water. A control with no added quinone was included in each experiment. Similarly prepared dark controls were included to insure that reduction of the dye occurred only in light. Upon addition of the chloroplast suspension to the reaction mixture, the absorptivity at 600 mμ was determined with a Beckman DU spectrophotometer. After 2 min at room temperature, the absorptivity was again determined, a 1 ml aliquot of the chloroplast suspension was extracted in 20 ml of methanol, and the chlorophyll content was determined spectrophotometrically at 578 mμ according to Vishniac.[17] The reaction rates are reported in units of absorptivity change per minute per milligram of chlorophyll.

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[7] W. VISHNIAC, in S. P. COLOWICK and N. O. KAPLAN, *Meth. Enzymol.* 4, 342 (1957).