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UNCOUPLING OF PHOTOSYNTHETIC PHOSPHORYLATION BY BENZOPHENANTHRIDINE ALKALOIDS

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SUMMARY

Sanguinarine, chelerythrine and chelidonine, benzophenanthridine alkaloids, inhibited both photosynthetic phosphorylation associated with ferricyanide reduction and cyclic photophosphorylation catalyzed by phenazine methosulphate. They did not affect electron transport in the presence of ADP and P_i and stimulated it in their absence. The inhibition of O_2 evolution by energy transfer inhibitors was reversed by the alkaloids. It is concluded that these alkaloids are uncouplers with the same efficiency in cyclic and non-cyclic photophosphorylation. This property might have some bearing in the physiological role of the alkaloids.

INTRODUCTION

The biological role of alkaloids, found primarily in higher plants, is not well understood but they probably behave as allelochemic agents[1] interfering with the metabolism of other organisms. One possible locus of their action may be the energy metabolism.

It has been shown recently, that some benzophenanthridine alkaloids are strong inhibitors of yeast respiration[2] and that low concentrations of one of them, chelerythrine, inhibit energy transfer in rat liver mitochondria and uncouple phosphorylation at higher concentrations[3]. Some of the effects of chelerythrine resemble those of Dio-9 (ref. 3), an antibiotic which, depending on the experimental conditions, may be an inhibitor of mitochondrial phosphorylation or an uncoupler[4], and which has also been found to be an effective inhibitor of photosynthetic phosphorylation[5].

The present paper shows that sanguinarine, chelerythrine and chelidonine, benzophenanthridine alkaloids, are powerful uncouplers of photophosphorylation in spinach chloroplasts.

RESULTS AND DISCUSSION

Fig. 1 shows that the benzophenanthridine alkaloids, sanguinarine, chelerythrine and chelidonine effectively inhibited photosynthetic phosphorylation associated with ferricyanide redution in spinach chloroplasts.

Electron transport from water to ferricyanide was not affected by the alkaloids

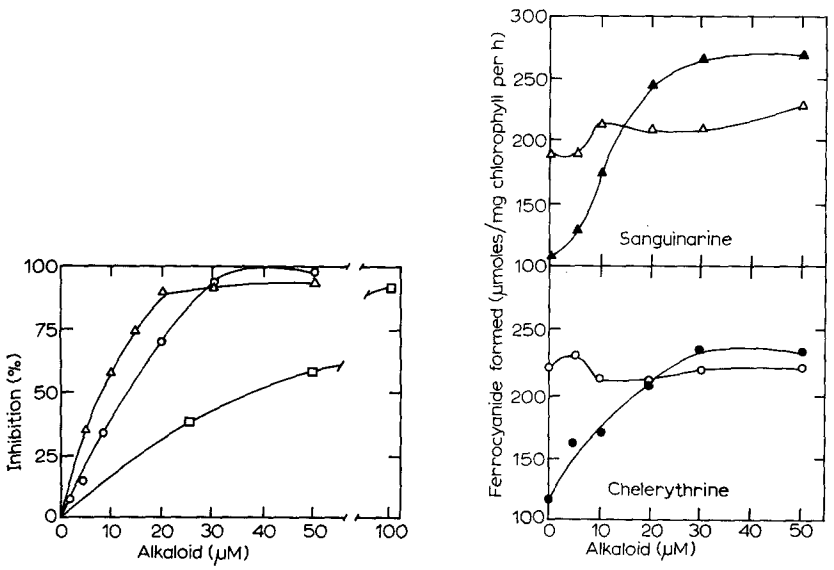

Fig. 1. Effect of benzophenanthridine alkaloids on photosynthetic phosphorylation associated with ferricyanide reduction in spinach chloroplasts. Experimental conditions were as described int he text. ATP synthesis in control tubes was 58 μmoles/mg of chlorophyll per h. △—△, sanguinarine chloride; ○—○, chelerythrine chloride; □—□, chelidonine.

Fig. 2. Effect of sanguinarine and chelerythrine on electron transport from water to ferricyanide. Experimental conditions were as described in the text. Open symbols, ADP and P_i were present. Closed symbols, ADP and P_i were absent.

in the presence of ADP and P_i but was considerably enhanced in their absence, as shown in Fig. 2 for sanguinarine and chelerythrine. O_2 evolution in the presence of light and ferricyanide was stimulated by the alkaloids as shown in Fig. 3A for chelerythrine. Chelerythrine also reversed the inhibition of O_2 evolution by energy transfer inhibitors like phlorizin (Fig. 3B) in the presence of ADP and P_i.

The cyclic photophosphorylation catalyzed by phenazine methosulphate was also inhibited by sanguinarine, chelerythrine and chelidonine (Fig. 4). The sensitivity of cyclic and non-cyclic photophosphorylation was the same with the three alkaloids. The I_{50} values for sanguinarine, chelerythrine and chelidonine were 9, 25 and 39 μM, respectively for ferricyanide associated phosphorylation and 7.5, 26 and 44 μM for cyclic photophosphorylation.

The results shown prove that sanguinarine, chelerythrine and chelidonine are uncouplers of photosynthetic phosphorylation since they permit or stimulate electron transport while inhibiting any associated ATP synthesis, and suggest that the alkaloids might be useful tools for the study of the mechanism of photophosphorylation.

Many different substances have been described as behaving as uncouplers of photosynthetic phosphorylation, among them amines and complex nitrogen bases[6] but to the best of my knowledge alkaloids have not been reported as uncouplers. The present results showing that some benzophenanthridine alkaloids uncouple photophosphorylation suggest a way in which they might act as allelochemic agents interfering with the growth of photosynthetic organisms[1].

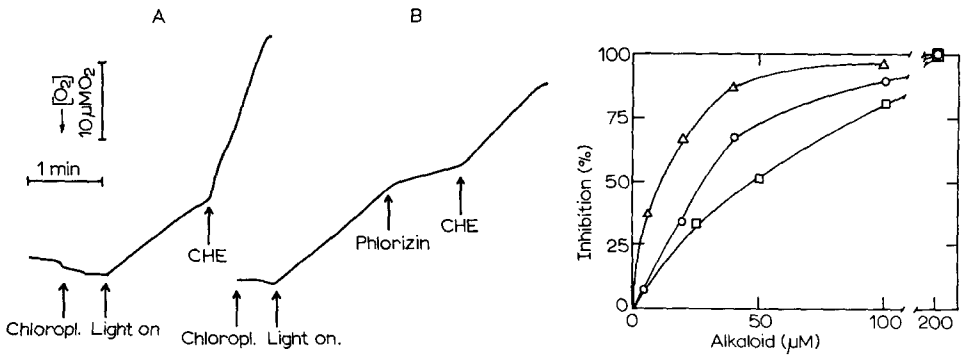

Fig. 3. Effect of chelerythrine on O_2 evolution. O_2 production was measured in a Gilson Oxygraph equiped with a Teflon-covered Clark oxygen electrode. The reaction medium (1.6 ml) was in Expt. A 250 mM sucrose, 25 mM *N*-tris(hydroxymethyl)methyl-2-aminomethanesulphonic acid–NaOH buffer (pH 7.8), 3 mM $MgCl_2$ and 1.2 mM $K_3Fe(CN)_6$ and Expt. B it was the same *plus* 2 mM ADP and 2 mM potassium phosphate. The reaction vessel was surrounded by a water bath at 25 °C and was illuminated by two 200-W tungsten lamps. Chloropl., chloroplasts (40 μg of chlorophyll); CHE, 31 μM chelerythrine. Phlorizin was, when added, 1 mM.

Fig. 4. Effect of benzophenanthridine alkaloids on cyclic photophosphorylation catalyzed by phenazine methosulphate. Experimental conditions were as described in the text. The control value for ATP synthesis was 750 μmoles/mg chlorophyll per h. △—△, sanguinarine; ○—○, chelerythrine; □—□, chelidonine.

EXPERIMENTAL

Chloroplasts were isolated from freshly collected spinach leaves as described by Nobel[7]. The isolation medium was 250 mM sucrose, 20 mM *N*-tris(hydroxymethyl)-methyl-2-aminoethanesulphonic acid)–NaOH buffer (pH 7.8) and 3 mM $MgCl_2$.

Photophosphorylation was determined in the same medium with the addition of 2 mM ADP, 2 mM potassium phosphate, $1 \cdot 10^6$ cpm carrier-free $^{32}P_i$ and either 1.2 mM $K_3Fe(CN)_6$ or 33 μM phenazine methosulphate. Incubations were carried out in small test tubes in a water bath at 25 °C in the dark or in saturating light provided by two 200-W tungsten lamps. The final volume of reaction medium was 1 ml, and contained an amount of chloroplasts equivalent to 10 μg of chlorophyll. The reaction was stopped after 3 min with 0.1 ml of 50% (w/v) trichloroacetic acid.

The $^{32}P_i$ incorporated into ATP was determined by an isobutanol–benzene extraction method[8] and counted in a Beckmann L.S-233 liquid scintillation counter[9]. The incorporation of $^{32}P_i$ in the dark was subtracted from that in the light.

Electron transport from water to ferricyanide was determined under similar experimental conditions omitting $^{32}P_i$ from the reaction medium. After the protein precipitate was removed by centrifugation, the ferrocyanide formed was determined in aliquots of supernatants as described by Avron and Shavit[10].

Total chlorophyll was determined as described by Whatley and Arnon[11].

Sanguinarine chloride was obtained from Koch-Light Laboratories (England), chelerythrine chloride from Pierce Chemical Co. (U.S.A.), and chelidonine from K and K Laboratories (U.S.A.). Alkaloid solutions were prepared in dimethyl-sulphoxide. The solvent was without effect at the concentrations used (less than 2%).

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