Biochimica et Biophysica Acta, 333 (1974) 141–148

BBA 46681

ALKALOIDS AS INHIBITORS OF PHOTOPHOSPHORYLATION IN SPINACH CHLOROPLASTS

RUBEN H. VALLEJOS and CARLOS S. ANDREO

Departamento de Bioquímica, Facultad de Ciencias Bioquímicas, Universidad Nacional de Rosario, Suipacha 531, Rosario (Argentina)

(Received August 9th, 1973)

SUMMARY

A group of 12 alkaloids were tested as inhibitors of photophosphorylation in spinach chloroplasts. Ajmaline, a dihydroindole alkaloid, was found to be the strongest inhibitor of both cyclic and non-cyclic photophosphorylation. Low concentrations of ajmaline also inhibited the dark and light ATPases, and the coupled electron flow from water to ferricyanide, measured either as ferrocyanide formed or as oxygen evolved, but not the uncoupled electron transport or the pH rise of illuminated unbuffered suspensions of chloroplasts. Higher concentrations of ajmaline stimulated, instead of inhibiting, photosynthetic electron transport or oxygen evolution and decreased the pH rise, thus behaving as an uncoupler, such as ammonia.

Photophosphorylation was partially inhibited by 100 μM dihydrosanguinarine, 100 μM dihydrochelerythrine (benzophenanthridine alkaloids); 500 μM *O,O'*-dimethylmagnoflorine, 500 μM *N*-methylcorydine (aporphine alkaloids) and 1 mM julocrotine. They also inhibited coupled oxygen evolution and only partially (dihydrosanguinarine and dihydrochelerythrine) or not at all (the other alkaloids) uncoupled oxygen evolution.

Spegazzinine (dihydroindole alkaloid), magnoflorine, *N*-methylisocorydine, coryneine (aporphine alkaloids), candicine and ribalinium chloride were without effect on photophosphorylation at 500 μM.

INTRODUCTION

Alkaloids are substances found mainly in higher plants and characterized by containing basic nitrogen. Little is known about the biochemical effects of alkaloids or their site of action as allelopathic agents [1] although there is an extensive literature on their pharmacological properties and on the rather specific ways by which some alkaloids are known to interfere with the physiology of animal or plant organisms. Only in a few cases have these observations been carried out at the molecular level [2].

Among the biochemical processes that may be affected by alkaloids, the possibility that the energetic metabolism may be the site of action of some of them has

received very little attention in the past. Recently, Vallejos and Roveri [3] reported that benzophenanthridine alkaloids among other alkaloids, were strong inhibitors of yeast cells respiration. The same alkaloids were found to inhibit oxidative phosphorylation in rat liver mitochondria [4] and to uncouple photosynthetic phosphorylation in spinach chloroplasts [5]. We reported recently [6] that a new peptide alkaloid, discarine B, specifically inhibited energy transfer reactions in chloroplasts.

The present paper describes the effects of some alkaloids, ajmaline, julocrotine, dihydrochelerythrine, dihydrosanguinarine, *O,O'*-dimethylmagnoflorine and *N*-methylcorydine on photophosphorylation in spinach chloroplasts and extends previous observations made with others [5, 6]. Ajmaline, a dihydroindole alkaloid [7] is currently being used as an anti arhythmic drug and its pharmacological properties have been studied [8]. Julocrotine is an alkaloid isolated from *Julocroton montevidensis* and has not a basic nitrogen but a secondary amide and imide groups [9]. Dihydrochelerythrine and dihydrosanguinarine are derivatives easily prepared from the benzophenanthridine alkaloids, chelerythrine and sanguinarine [10] and sometimes found in the same plants [11]. *O,O'*-Dimethylmagnoflorine and *N*-methylcorydine are aporphine alkaloids found in *Fagara* spp. [12].

It is shown that all of them inhibit photosynthetic phosphorylation, ajmaline being the most effective inhibitor.

RESULTS AND DISCUSSION

Photosynthetic phosphorylation in spinach chloroplasts was completely inhibited by the dihydroindole alkaloid, ajmaline (Fig. 1). Both cyclic and non-cyclic

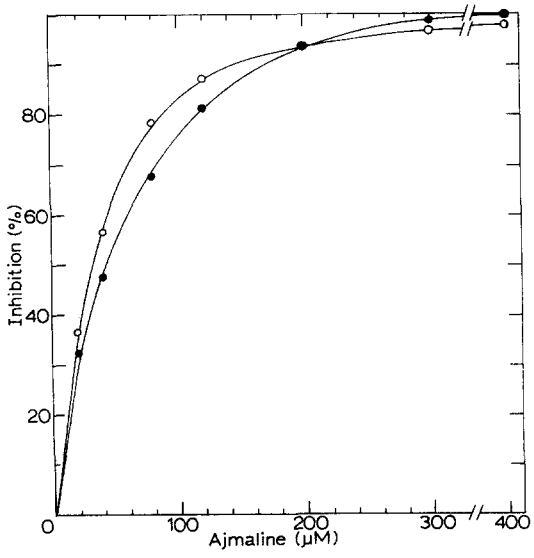

Fig. 1. Inhibition of cyclic and non-cyclic photophosphorylation in spinach chloroplasts by ajmaline. Cyclic phosphorylation catalized by phenazine methosulphate and non-cyclic phosphorylation with ferricyanide as electron acceptor were determined as described in the text. The control rates of cyclic (○–○) and non-cyclic (●–●) photophosphorylation were 221.3 and 40.7 μmoles ATP/mg chlorophyll per h, respectively.

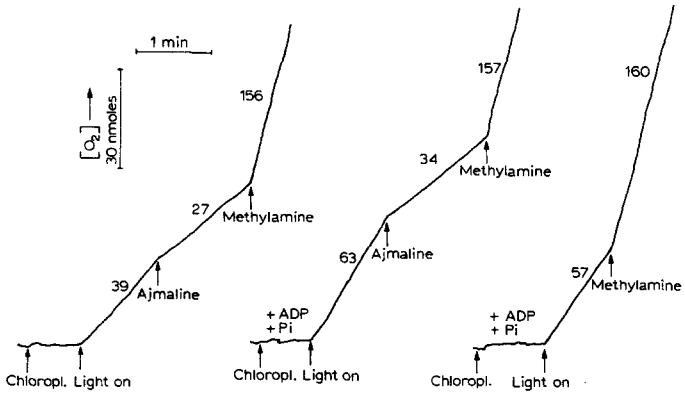

Fig. 2. Effect of ajmaline on oxygen generation. Oxygen productions was measured in a Gilson oxygraph equiped with a Teflon-covered Clark oxygen electrode. The reaction medium (1.65 ml) was 250 mM sucrose, 20 mM *N*-tris(hidroxymethyl)methyl-2-amino-ethanesulphonic acid–NaOH buffer (pH 7.8), 3 mM $MgCl_2$ and 1.2 mM ferricyanide. The reaction vessel was surrounded by a water-bath a 25 °C and was illuminated by two 200-W tungsten lamps. Chloropl., chloroplasts (40 μg of chlorophyll). Ajmaline, was when added, 80 μM; methylamine chloride, 10 mM; ADP, 2 mM and P_i (potassium phosphate) 2 mM. The numerals on the slopes are μmoles oxygen/mg chlorophyll per h.

photophosphorylation were inhibited to the same extent by the alkaloid. The I_{50} (the concentration producing 50 % of inhibition) was about 40 μM for both processes. Oxygen evolution by iluminated choroplasts with ferricyanide as electron acceptor was inhibited by low concentrations of ajmaline, specially in the presence of ADP and P_i but the inhibition was completely released by uncouplers such as methylamine (Fig. 2). The inhibition of oxygen evolution in the absence of ADP and P_i was similar to that observed with Dio-9 and discarine B which has been explained as due to some degree of coupling of the "basal" electron flow [13]. Higher concen-

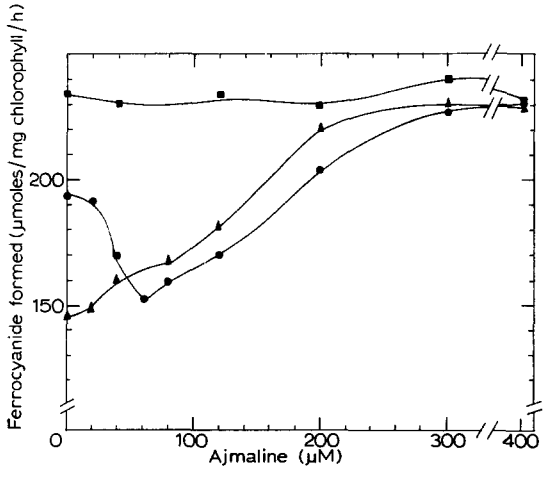

Fig. 3. Effect of ajmaline on electron transport from water to ferricyanide. Experimental conditions were as described in the text. ▲–▲. ADP and P_i were absent; ●–●, ADP and P_i were present; ■–■, ADP, P_i and 10 mM methylamine chloride were present.

trations of ajmaline did not inhibit photosynthetic electron transport from water to ferricyanide but stimulated it (Fig. 3). The inhibition of coupled electron transport by low concentrations of ajmaline was consistently observed in six experiments. Uncoupled electron flow, measured as ferrocyanide formed (Fig. 3), was not affected by alkaloid concentrations up to 400 μM.

These results suggest that ajmaline behaves as an energy transfer inhibitor at low concentrations (up to 60 or 80 μM) which inhibited about 70 % of photophosphorylation, and as an uncoupler at higher concentrations.

Neumann and Jagendorf [14] have shown that there is an increase in pH of an unbuffered suspension of chloroplasts upon illumination which has been correlated with photophosphorylation. Various uncouplers inhibited the pH change whereas energy transfer inhibitors like Dio-9, phloridzin, dicyclohexylcarbodiimide and chlorotri-*n*-butyltin did not affect the light-induced proton gradient [15–18]. The latter two enhanced the light-induced pH rise in EDTA-treated chloroplasts [17, 18].

The effect of ajmaline on the pH of illuminated unbuffered suspensions of chloroplasts confirmed the observed biphasic behavior of the alkaloid 40 μM ajmaline which inhibited 50 % photophosphorylation and inhibited the associated electron transport did not affect the pH rise of illuminated chloroplasts as expected of an energy transfer inhibitor like Dio-9 (Ref. 15, Table 1). Discarine B, a recently described energy transfer inhibitor [6], was similarly ineffective. Higher concentrations of ajmaline (200 μM) which not only almost completely inhibited photophosphorylation (Fig. 1) but stimulated electron transport instead of decreasing it (Fig. 3), inhibited the light-induced proton gradient as expected of uncouplers like atebrine [14] (Table I). The same effect, inhibition of the pH rise, was also observed with the benzophenanthridine alkaloids, sanguinarine and chelerythrine (Table I), which have been described as uncouplers of photophosphorylation [5]. As such they stimulated oxygen evolution (Table II, Expts 2 and 7). Their dihydro-derivatives dihydrochelerythrine and dihydrosanguinarine did not affect the pH rise (Table I) and inhibited instead of stimulating, oxygen generation by chloroplasts (Table II,

TABLE I

EFFECT OF ALKALOIDS ON THE LIGHT-INDUCED pH RISE

Experimental conditions were as described in the text. The total increase in pH of the solution after turning the light on is given. Each value represents the mean of duplicate runs.

Additions	Total pH rise
None	0.101
Ajmaline (40 μM)	0.100
Ajmaline (200 μM)	0.045
Sanguinarine (40 μM)	0.045
Chelerythrine (60 μM)	0.060
Atebrine (50 μM)	0.030
Dio-9 (10 μg/ml)	0.095
Discarine B (100 μM)	0.100
Dihydrosanguinarine (50 μM)	0.100
Dihydrochelerythrine (100 μM)	0.101

Expts 3 and 8). The inhibition was partially released by chelerythrine, sanguinarine or methylamine (Table II, Expts 4, 9 and 6). The dihydroderivatives inhibited both cyclic and non-cyclic photophosphorylation to the same extent but at higher concentrations than chelerythrine and sanguinarine. For example, I_{50} for sanguinarine and chelerythrine were 9 and 25 μM, respectively (ref. 5), while 100 μM dihydrosangui-

TABLE II

EFFECT OF ALKALOIDS ON OXYGEN EVOLUTION BY SPINACH CHLOROPLASTS

Experimental conditions were as described in the legend to Fig. 2. ADP and P_i were, when present, 2 mM.

Expt	Additions	Oxygen evolution (μmoles/mg chlorophyll per h)	
		+ADP+P_i	−ADP−P_i
1	None	51.8	28.7
2	Chelerythrine (62.5 μM)	–	61.0
3	Dihydrochelerythrine (62.5 μM)	21.8	14.9
4	Expt 3 plus chelerythrine (62.5 μM)	38.0	42.5
5	Methylamine (10 mM)	113.6	115.0
6	Expt 3 plus methylamine (10 mM)	73.6	86.3
7	Sanguinarine (62.5 μM)	–	46.0
8	Dihydrosanguinarine (62.5 μM)	–	14.9
9	Expt 8 plus sanguinarine (62.5 μM)	–	32.2

TABLE III

SUMMARY OF THE EFFECTS OF ALKALOIDS ON ELECTRON TRANSPORT, PHOSPHORYLATION AND pH RISE

Experimental conditions were as described in the text. Controls values for cyclic and non-cyclic phosphorylation were 50–60 μmoles ATP/mg chlorophyll per h and 180–210 μmoles ATP/mg chlorophyll per h, respectively.

Alkaloid (μM)	Effect of Alkaloid on				
	Electron transport		Phosphorylation (% inhibition)		pH rise
	Coupled	Uncoupled	Cyclic	Non-cyclic	
Ajmaline (80)	Inhibited	No effect	70	70	No effect
Ajmaline (200)	Stimulated	No effect	90	90	Inhibited
Sanguinarine (40)	No effect*	–	100(ref. 5)	95(ref. 5)	Inhibited
Chelerythrine (60)	No effect*	–	90(ref. 5)	80(ref. 5)	Inhibited
Dihydrosanguinarine (100)	Inhibited	Partially inhibited	65	65	No effect
Dihydrochelerythrine (100)	Inhibited	Partially inhibited	60	60	No effect
Discarine B (100)	Inhibited (ref. 6)	No effect (ref. 6)	80(ref. 6)	80(ref. 6)	No effect
O,O'-Dimethylmagnoflorine (500)	Inhibited	No effect	40	–	–
N-Methylcorydine (1000)	Inhibited	No effect	61	–	–
Julocrotine (1000)	Inhibited	No effect	50	50	–

* Sanguinarine and chelerythrine stimulated basal electron transport [5].

narine inhibited the non-cyclic phosphorylation by 65 % and 100 μM dihydrochelerythrine by 60 % (Table III). These results show that the dihydro-derivatives may be classified as mixed-type inhibitors of photophosphorylation like dicyclohexylcarbodiimide [17] instead of as uncouplers.

Phosphorylation associated with ferricyanide reduction and coupled electron transport were also inhibited by the aporphine alkaloids, *O,O'*-dimethylmagnoflorine and *N*-methylcorydine and by julocrotine (Table III) whereas the uncoupled electron transport was not affected. Therefore, these alkaloids exerted their effects on the energy transfer system. The dihydroindole alkaloid, spegazzinine, the aporphine alkaloids magnoflorine, *N*-methylisocorydine and coryneine, candicine and ribalinium chloride were without effect on photophosphorylation at 500 μM.

A dark ATPase and several light-induced ATPases have been described in chloroplasts [19]; all of them seem to represent variations of the reversal of the reactions leading to the synthesis of ATP in photophosphorylation [19]. The dark ATPase activity of chloroplasts has been claimed to be non-existing [19] but Kraayenhof et al. [20] have shown that photophosphorylation is reversible in the dark and in the absence of sulfhydryl reagents. Both ATPase activities are inhibited by uncouplers and inhibitors of photophosphorylation [20].

Table IV shows that 80 μM ajmaline inhibited both the dark and the light ATPase activities of chloroplasts. Higher concentrations (200 μM) of ajmaline did not further increase the inhibition. The ATPases were also inhibited by 100 μM dihydrosanguinarine, 100 μM dihydrochelerythrine, 400 μM discarine B, 1 mM julocrotine and, as previously described [21, 22] by Dio-9 and phloridzin (Table IV).

In conclusion 10 alkaloids out of 16 studied here inhibited photophosphorylation (Table III). These and previous results [4–6] show that some alkaloids are strong and specific inhibitors of bioenergetic processes. Their known chemical structure and the possibility of modifying it may lead to stronger and even more specific inhibitors that may help to unravel the mechanism of energy conservation.

TABLE IV

EFFECT OF ALKALOIDS ON THE DARK AND LIGHT ATPase ACTIVITIES OF CHLOROPLASTS

Experimental conditions were as described in the text.

Expt	Additions	ATPase activity (μmoles Pi/mg chlorophyll per h)	
		Dark	Light
1	None	7.00	13.53
	Ajmaline (80 μM)	4.35	5.41
	Ajmaline (200 μM)	4.35	5.07
	Dihydrosanguinarine (100 μM)	3.39	4.05
	Julocrotine (1 mM)	4.10	4.06
	Dio-9 (10 μg/ml)	4.35	5.07
2	None	6.29	13.21
	Dihydrochelerythrine (100 μM)	5.32	5.42
	Discarine B (400 μM)	4.36	1.35
	Phloridzin (2 mM)	4.36	2.03

The interference of some alkaloids with the energetic metabolism may be relevant to their postulated allelopathic role [1] specially considering that the target organism may be more sensitive than those used in this and previcus studies [4–6] or that it may accumulate the alkaloid.

EXPERIMENTAL

Chloroplasts were isolated from spinach leaves (*Spinacea olearacea* L) as previously described [5].

Photophosphorylation and electron transport from water to ferricyanide were determined as described [5]. The reaction medium (1 ml) was 250 mM sucrose, 20 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid–NaOH buffer (pH 7.8), 3 mM $MgCl_2$ and either 1.2 mM ferricyanide or 33 μM phenazine methosulphate. ADP and potassium phosphate were 2 mM when added. An amount of chloroplasts equivalent to 10 μg of chlorophyll was used per test tube.

Total chlorophyll was determined as described [23].

The pH change of chloroplasts suspensions was measured with a Beckman research pH meter equipped with a combination electrode and a recorder. The reaction medium (3 ml) was 50 mM KCl, 20 μM pyocyanine and chloroplasts containing 120 μg of chlorophyll, prepared as usual but suspended in unbuffered 50 mM KCl. The pH was adjusted to 6.40. The reaction vessel was surrounded by a water-bath at 25 °C containing a trace of $CuSO_4$ for alliviating the heating effects of the light [24], and was illuminated by two 200-W tungsten lamps.

The dark ATPase was determined in a reaction medium (1 ml) containing 250 mM sucrose, 20 mM *N*-tris (hydroxymethyl)methyl-2-aminoethanesulphonic acid NaOH buffer (pH 7.8), 3 mM $MgCl_2$ and chloroplasts containing 65 μg of chlorophyll. Temperature was 30 °C. After 2 min of preincubation the reaction was started by adding 3 μmoles ATP and stopped after 15 min with 0.1 ml of trichloroacetic acid (50 % w/v). P_i was determined according to Sumner [25].

The light-triggered ATPase was determined in the same medium with the addition of 5 mM dithioerythritol and the reaction was started after preincubation in saturating light for 2 min.

Sanguinarine chloride, chelerythrine chloride, and phloridzin were obtained from Koch–Light Laboratories (England), Pierce Chemicals Co. (U.S.A.) and Nutritional Biochemical Corp. (U.S.A.), respectively. Ajmaline, julocrotine, spegazzinine and ribalinium chloride were gifts of the División de Química Orgánica Superior, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Dio-9 was a gift of Dr B. Kemp, University of Amsterdam (The Netherlands). Dihydrosanguinarine and dihydrochelerythrine were gifts of Dr Giacopello, Departamento de Química Orgánica, Facultad de Ciencias Exactas, Universidad de Buenos Aires or were prepared as described [10]. Discarine B was a gift of Dr O. A. Mascaretti, and all the others alkaloids tested were gifts of Dr Rúveda and Dr Kuck, from the Departamento de Química Orgánica, Facultad de Bioquímica y Farmacia, Universidad de Buenos Aires. Alkaloids solutions were prepared in dimethylsulphoxide. The solvent at the final concentrations used (less than 2 %) and the accompanying anions of some of the alkaloids (chloride, iodide, fumarate or oxalate) were without effect in control experiments.

ACKNOWLEDGEMENTS

We wish to thank Dr F. Seta for allowing the use of equipment belonging to his Department. R.H.V. is a Career Investigator and C.S.A. is a Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina. This work was supported by grants from the same institution and the Consejo de Investigaciones de la Universidad Nacional de Rosario, Argentina.

REFERENCES

1 Whittaker, R. H. and Feeny, P. P. (1971) Science 171, 757–770
2 Robinson, T. (1968) Biochemistry of Alkaloids, 1st edn, pp.125–135, Springer-Verlag, New York
3 Vallejos, R. H. and Roveri, O. A. (1972) Biochem. Pharmacol. 21, 3179–3182
4 Vallejos, R. H. and Rizzotto, M. G. (1972) FEBS Lett. 21, 195–198
5 Vallejos, R. H. (1973) Biochem. Biophys. Acta 292, 193–196
6 Andreo, C. S. and Vallejos, R. H. (1973) FEBS Lett. 33, 201–204
7 Woodward, R. B. (1956) Angew. Chem. 68, 13–20
8 Mela, C. (1967) Fitoterapia 38, 2–15
9 Nakano, T., Djerassi, C., Corral, R. A. and Orazi, O. O. (1961) J. Org. Chem. 26, 1184–1191
10 Labriola, R. A. and Giacopello, D. (1966) An. Asoc. Quím. Argent. 45, 15–27
11 Scheuer, P. J., Chang, M. I. and Swanholm, C. E. (1962) J. Org. Chem. 27, 1472–1473
12 Kuck, A. M., Albónico, S. M., Delofeu, V. and Escalante, M. G. (1967) Phytochemistry 6, 1541–1550
13 Gromet-Elhanan, Z. (1968) Arch. Biochem. Biophys. 123, 447–456
14 Neumann, J. and Jagendorf, A. T. (1964) Arch. Biochem. Biophys. 107, 109–119
15 McCarty, R. E. and Racker, E. (1967) Brookhaven Symp. Biol. 19, 202–214
16 Crofts, A. R. (1967) J. Biol. Chem. 242, 3352–3359
17 McCarty, R. E. and Racker, E. (1967) J. Biol. Chem. 242, 3435–3439
18 Khan, J. S. (1968) Biochim. Biophys. Acta 153, 203–210
19 Avron, M. and Neumann, J. (1968) Annu. Rev. Plant Physicol. 19, 137–166
20 Kraayenhof, R., Groot, C. S. P. and Van Dam, K. (1970) Electron Transport and Energy Conservation (Tager, J. M., Papa, A., Quagliarello, E. and Slater, E. C., eds), pp. 201–214, Adriatica Editrice, Bari
21 McCarty, R. E. and Racker, E. (1968) J. Biol. Chem. 243, 129–137
22 Carmeli, C. (1969) Biochim. Biophys. Acta 189, 256–266
23 Whatley, F. R. and Arnon, D. I. (1963) Methods in Enzymology (Colowick, S. P. and Kaplan, N. O., eds), Vol. 2, pp. 308–313, Academic Press, New York
24 Dilley, R. A. (1972) Methods in Enzymology (San Pietro, A., ed.), Vol. 24, Part B, pp. 68–72, Academic Press, New York
25 Sumner, J. B. (1944) Science 100, 413–414