INHIBITION OF ENERGY TRANSFER REACTIONS IN SPINACH CHLOROPLASTS BY DISCARINE B, A NEW PEPTIDE ALKALOID

C.S. ANDREO and Ruben H. VALLEJOS

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

Received 22 April 1973

1. Introduction

Specific inhibitors are very useful tools for the study of complex biochemical processes like oxidative phosphorylation or photosynthetic phosphorylation. Phlorizin [1] and Dio-9 [2] are well known inhibitors of energy transfer reactions in photophosphorylation.

Peptide alkaloids are a recently recognized group of polyamide plant bases composed largely of simple amino acids [3]. Very little is known about their biological or pharmacological properties [4].

The present paper describes the effect on photosynthetic phosphorylation of two new peptide alkaloids, discarines A and B obtained from *Discaria longispina* [5], a rhamnaceous Argentinian plant known to be used in folk medicine. One of these peptide alkaloids, discarine B, is shown to be a specific inhibitor of energy transfer in spinach chloroplasts while discarine A behaves as a mixed-type inhibitor.

2. Experimental

Chloroplasts were isolated from freshly collected spinach leaves as described by Nobel [6]. The isolation medium was 250 mM sucrose, 20 mM N-tris(hydroxymethyl)-methyl-2-amino ethanesulphonic acid)-NaOH buffer (pH: 7.8) and 3 mM $MgCl_2$.

Photophosphorylation and electron transport from water to ferricyanide were determined as described by Vallejos [7]. Ferrocyanide formation was determined as described by Avron and Shavit [8].

Total chlorophyll was determined as described by Whatley and Arnon [9].

Peptide alkaloids (bases or furmarate salts) solutions were freshly prepared in dimethylsulphoxide. The fumarate and the solvent were without effect at the concentrations used (less than 2% for the solvent).

3. Results and discussion

Fig. 1 shows the titration curves of the inhibitory effect of discarines A and B on non-cyclic photophosphorylation associated with ferricyanide reduction in spinach chloroplasts. Discarine B was the most effective inhibitor.

Cyclic photophosphorylation catalyzed by phenazine methosulphate was also inhibited by the peptide alkaloids (fig. 2). Cyclic and non-cyclic photophosphorylation were equally sensitive to the inhibition by these peptide alkaloids, The I_{50} values for discarine B, and discarine A were 55 and 230 μM, respectively.

The described inhibition of photophosphorylation may be explained by an effect of the alkaloids on either the electron transport or the energy transfer reactions. Fig. 3A shows that 125 μM discarine B inhibited oxygen evolution by illuminated chloroplasts in the presence of ferricyanide, ADP and P_i. The inhibition was totally reversed by 10 mM methylamine, a known uncoupler of photophosphorylation (see fig. 3C). There was also some inhibition (29%) of oxygen evolution by discarine B in the absence of ADP and P_i (fig. 3B) but it was also reversed by methylamine. Similar effects were observed with 250 μM discarine A (fig. 3D and E). Other uncouplers like NH_4Cl (fig. 3D and F) were also able to reverse the inhibition of oxygen evolution by the peptide alkaloids.

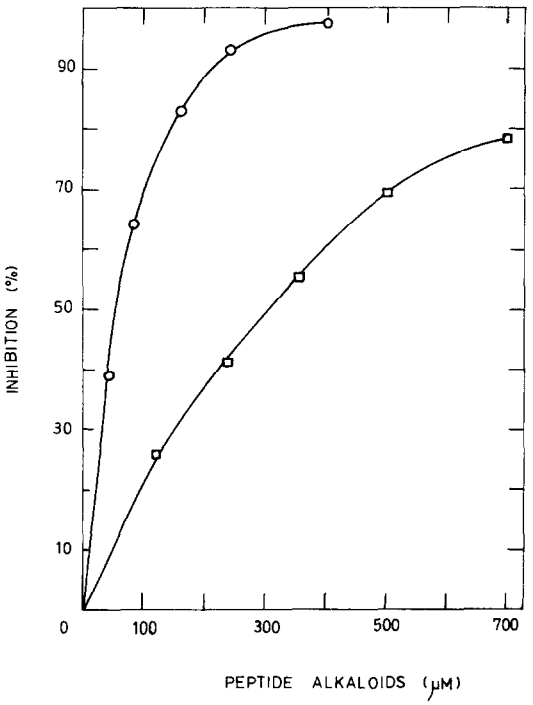

Fig. 1. Effect of discarines A and B on non-cyclic photophosphorylation in spinach chloroplasts. ATP synthesis in control tubes was 42 μmoles/mg chlorophyll per hr. (○—○—○) Discarine B; (□—□—□) discarine A.

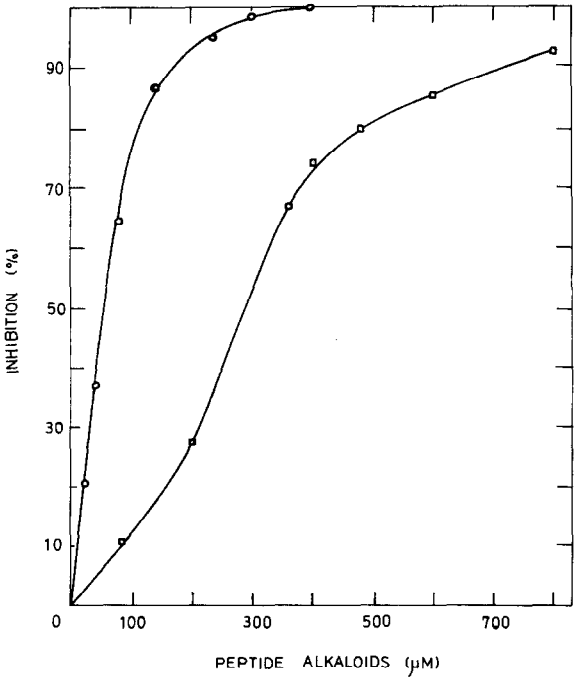

Fig. 2. Effect of discarines A and B on cyclic photophosphorylation catalyzed by phenazine methosulphate in spinach chloroplasts. Experimental conditions were as described in the text. ATP synthesis in control tubes was 231 μmoles/mg chlorophyll per hr. (○—○—○) Discarine B; (□—□—□) discarine A.

According to the definition of Izawa and Good [10] a typical energy transfer inhibitor should not inhibit photosynthetic electron transport in the presence of an uncoupler. That is exactly the case with discarine B (fig. 4). Within the range of concentration that completely inhibited cyclic and non-cyclic photophosphorylation, discarine B did not affect the photosynthetic reduction of ferricyanide in the presence of methylamine or in the absence of ADP and P_i (fig. 4) whereas it inhibited the increased rate of ferricyanide reduction due to the addition of ADP and P_i.

Discarine A up to 250 μM behaved like discarine B (fig. 5) but at higher concentrations it drastically inhibited electron transport even in the presence of uncouplers.

The structures of discarines A and B are known [5]. They are isomers that differ in the positions of a β-indolylmethyl and a *sec*-butyl groups.

Frangulanine, a closely related compound obtained also from *Discaria longispina* [5], was found to inhibit photophosphorylation like discarine A, but inhibition of uncoupled electron transport was observed above 500 μM frangulanine, a concentration that inhibited nearly 90% of non-cyclic photophosphorylation.

In conclusion the results described here show that discarine B is a typical energy transfer inhibitor in photophosphorylation. The partial inhibition of oxygen evolution in the absence of ADP and P_i (fig. 3B) is similar to the inhibition by Dio-9 [2] and both may be explained by endogenous ADP generation (see [11]).

On the other hand discarine A is a mixed-type inhibitor behaving like an energy transfer inhibitor at low concentrations and like an electron transport inhibitor at higher concentrations.

The described effects of these peptide alkaloids may have some bearing on their biological properties in line with the proposed role of alelochemical agents [7, 12].

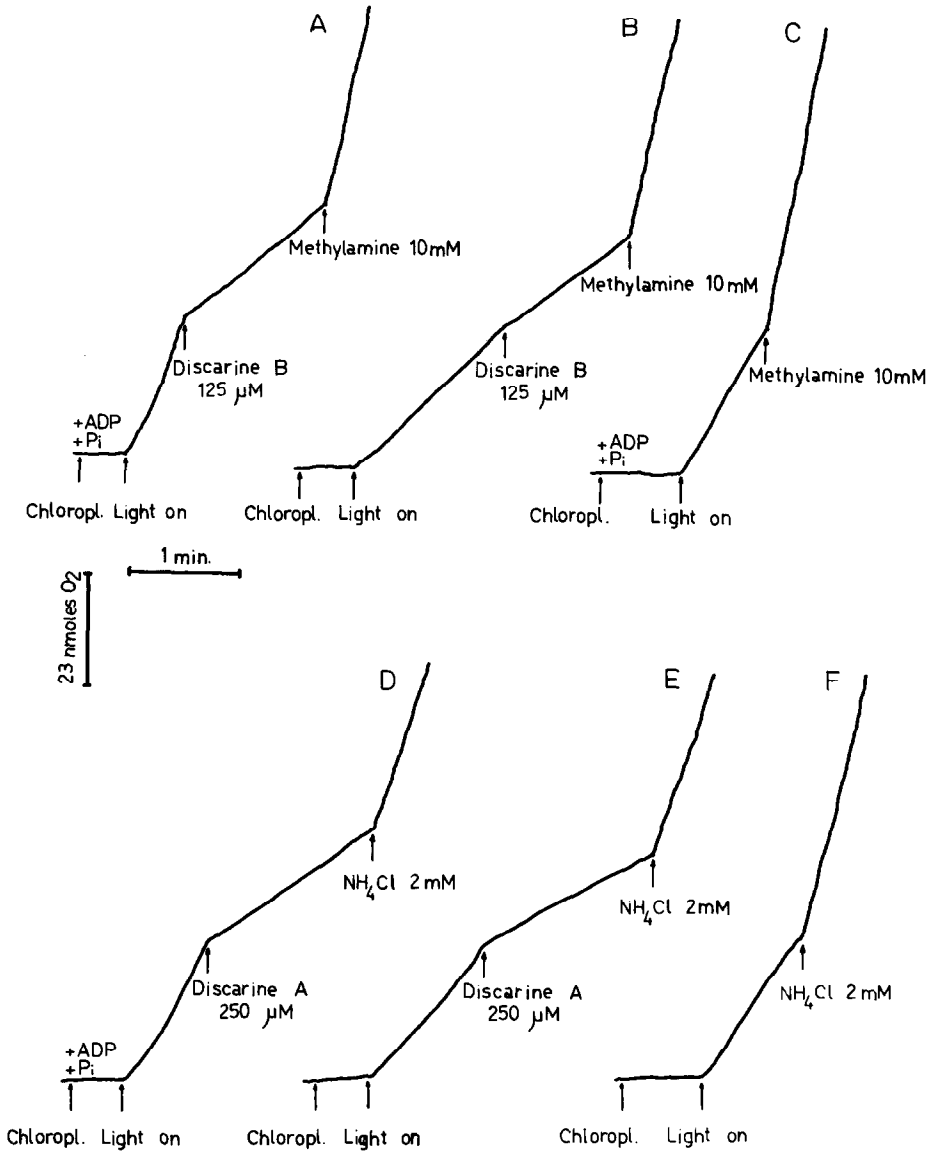

Fig. 3. Effect of discarines A and B on oxygen evolution in spinach chloroplasts. Oxygen generation was measured with a Teflon-covered Clark oxygen electrode in a Gilson Oxygraph. The reaction medium (1.65 ml) was 250 mM sucrose, 25 mM *N*-tris(hydroxymethyl)methyl-2-aminomethanesulphonic acid-NaOH buffer (pH: 7.8), 3 mM $MgCl_2$ and 1.2 mM $K_3Fe(CN)_6$. The reaction vessel was thermostatically controlled at 250°C and illuminated with 2 reflector flood lamps of 150 W each. Chlorpl., chloroplasts (40 μg of chlorophyll) when added ADP was 2 mM; P_i, potassium phosphate 2 mM.

Acknowledgements

We wish to thank Dr. O.A. Mascaretti (Department of Organic Chemistry, University of Buenos Aires) for the generous gift of the peptide alkaloids.

R.H.V. is a Career Investigator and C.S.A. is a Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

This work was supported by grants from CONICET and Consejo de Investigaciones Científicas y Técnicas de la Provincia de Santa Fe (Argentina).

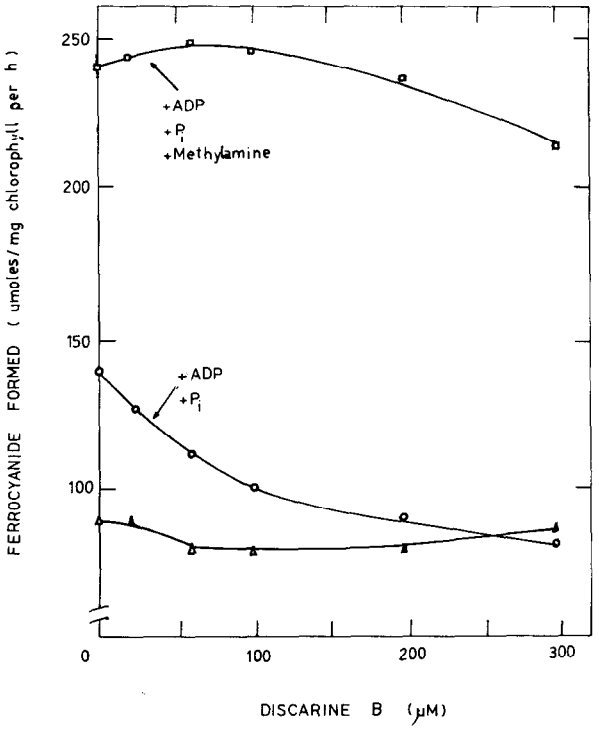

Fig. 4. Effect of discarine B on electron transport from water to ferricyanide in spinach chloroplasts. Experimental conditions were as described in the text and in the legend to fig. 3.

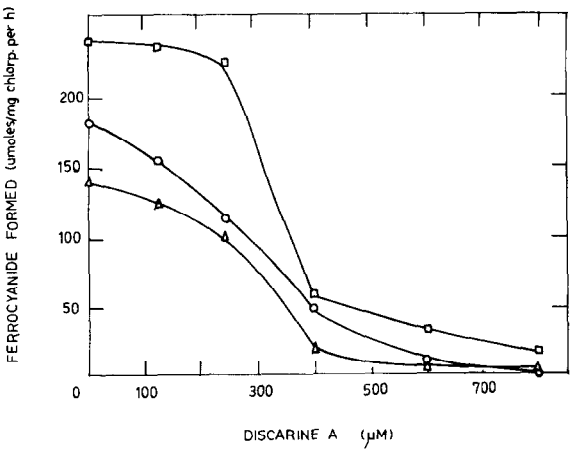

Fig. 5. Effect of discarine A on electron transport from water to ferricyanide in spinach chloroplasts. Experimental conditions were as described in the text and in the legend to fig. 3.

References

[1] S. Izawa, D.G. Winget and N.E. Good, Biochem. Biophys. Res. Commun. 22 (1966) 223.
[2] R.E. McCarty, R.J. Guillory and E. Racker, J. Biol. Chem. 240 (1965) PC 4822.
[3] E.W. Warnhoff, Specialist Periodical Reports No. 6, The Chemical Society, London, 1 (1971) 444.
[4] E.W. Warnhoff, Fortschr. Chem. Org. Naturstoffe 28 (1970) 162.
[5] O.A. Mascaretti, V.M. Merkuza, G.E. Ferraro, E.A. Rúveda, C.-J. Chang and E. Wenkert, Phytochem. 11 (1972) 1133.
[6] P.S. Nobel, Plant. Physiol. 42 (1967) 1389.
[7] R.H. Vallejos, Biochim. Biophys. Acta 292 (1973) 193.
[8] M. Avron and N. Shavit, Anal. Biochem. 6 (1963) 549.
[9] F.R. Whatley and D.I. Arnon, in: Methods in enzymology, Vol. 2, eds. S.P. Colowick and N.O. Kaplan (Academic Press, New York, N.Y., 1963) p. 308.
[10] S. Izawa and N.E. Good, in: Methods in enzymology, Vol. 24, ed. A. San Pietro (Academic Press, New York and London, 1972) p. 355.
[11] Z. Gromet-Elhanan, Arch. Biochem. Biophys. 123 (1968) 447.
[12] R.H. Whittaker and P.P. Feeny, Science 171 (1971) 757.