Uncoupling of Spinach Thylakoids by Gramine

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The indol alkaloid gramine inhibited photophosphorylation, Pi-ATP exchange reaction and proton gradient, and enhanced electron transport in spinach thylakoids with I_{50} around 0.2 mM. It thus behaves as a typical uncoupler of photophosphorylation.

Introduction

Gramine and related alkaloids isolated from fodder plants in the Gramineae, Leguminoseae and other plant families [1] are responsible for the toxicity observed in ruminants grazing on these plants [2, 3]. Recently, these alkaloids were reported as toxic to aphids feeding on graminaceous crops [4]. Toxicity of gramine has been related to the known physiological effects of tryptamine related compounds [5]. However, a report on the effects of gramine on energy metabolism of mitochondria [6], provided an alternate mechanism to its toxic action. Further insight into the nature of these effects can be gained through a study of the effects of gramine on energy transduction in thylakoids. We herein report such study. This type of study has been used to assess the importance as allelochemicals of other indol and peptide alkaloids [7–9].

Materials and Methods

Chloroplasts were isolated from market spinach leaves (*Spinacea olearacea* L.) as previously described [8] and suspended, unless otherwise indicated, in 250 mM sucrose, 20 mM Tris-HCl (pH 7.8) and 5 mM $MgCl_2$.

Cyclic photophosphorylation catalyzed by phenazine methosulfate, noncyclic photophosphorylation, and electron transport from water to methylviologen [8], the pH change of thylakoid suspensions [9], the Pi-ATP exchange reaction [10], and total chlorophyll [11] were determined as described in literature.

Gramine (obtained from Sigma Chemical Co.) was recrystallized once from acetone before use. Further recrystallizations did not change its behaviour as a single compound as judget by u · v spectra and by thin layer chromatograms using different elution solvent mixtures. Gramine was dissolved in dimethylsulfoxide. Controls with the solvent (less than 2%) were performed for all the reaction used.

Results and Discussion

Photosynthetic phosphorylation in spinach thylakoid was completely inhibited by the indol alkaloid gramine (Fig. 1). Both cyclic photophosphorylation catalyzed by phenazine methosulfate and non-cyclic photophosphorylation associated with methylviologen reduction were completely inhibited by the alkaloid. The I_{50} (concentration producing 50% inhibition) was about 200 μM for both processes, although cyclic photophosphorylation was slightly more sensitive.

The light-dependent synthesis of ATP by illuminated thylakoids may be inhibited in a number of ways: a) by blocking the electron transport, b) by uncoupling ATP synthesis from the electron transport, and c) by blocking the phosphorylation reaction itself. Reagents that block electron transport also inhibit ATP synthesis since the generation of the transmembrane electrochemical gradient, the driving force for ATP synthesis is dependent upon electron flow. Chemicals that increased the proton permeability of thylakoid membranes uncouple phosphorylation from electron flow. Uncoupling agents inhibit ATP synthesis by decreasing the proton gradient but allow electron transport to occur

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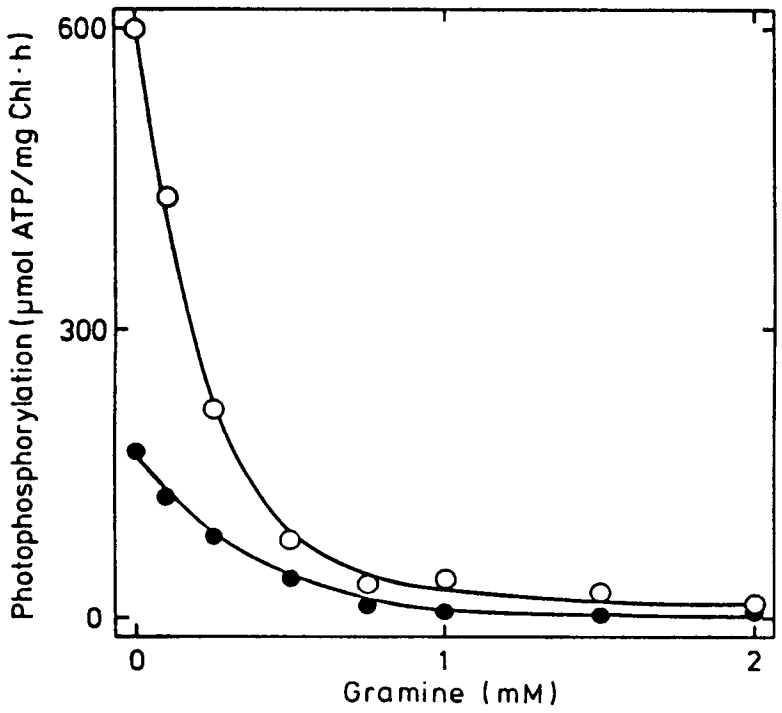

Fig. 1. Inhibition of cyclic and non-cyclic photophosphorylation in spinach thylakoid by gramine. Cyclic photophosphorylation (○) catalyzed by phenazine methosulfate and non-cyclic photophosphorylation (●) with methylviologen as electron acceptor were determined as described in the text. The reaction medium (1 ml) was 250 mM sucrose, 20 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 2 mM ADP, 3 mM potassium phosphate containing 1×10^6 cpm of ^{32}P, and either 50 μM phenazine methosulfate or 100 μM methylviologen and 500 μM NaN_3. An amount of thylakoids equivalent to 10 μg of chlorophyll was used per test tube.

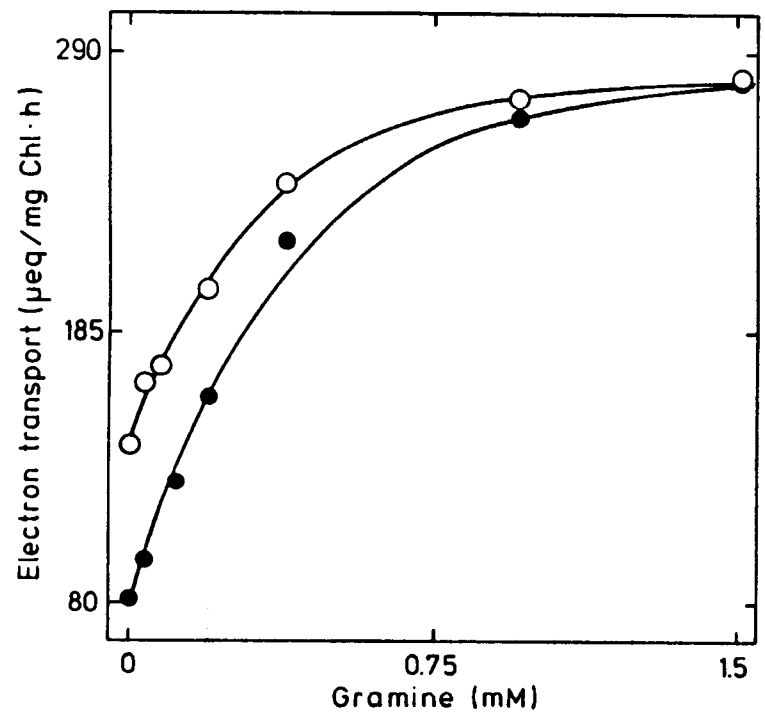

Fig. 2. Effect of gramine on electron transport. Photosynthetic electron transport from water to methylviologen was measured as oxygen uptake with a Teflon-covered Clark electrode. The reaction medium (1.65 ml) was 250 mM sucrose, 20 mM Tris-HCl (pH 7.8), 5 mM $MgCl_2$, 100 μM methylviologen, 500 μM NaN_3, and thylakoids (40 μg of chlorophyll). The reaction vessel was surrounded by a water bath at 25 °C and was illuminated by two 150-W tungsten lamps. The electron transport was measured in basal conditions (●) in the absence of ADP and Pi, and in coupled conditions (○) *i.e.* in the presence of 2 mM ADP and 3 mM Pi.

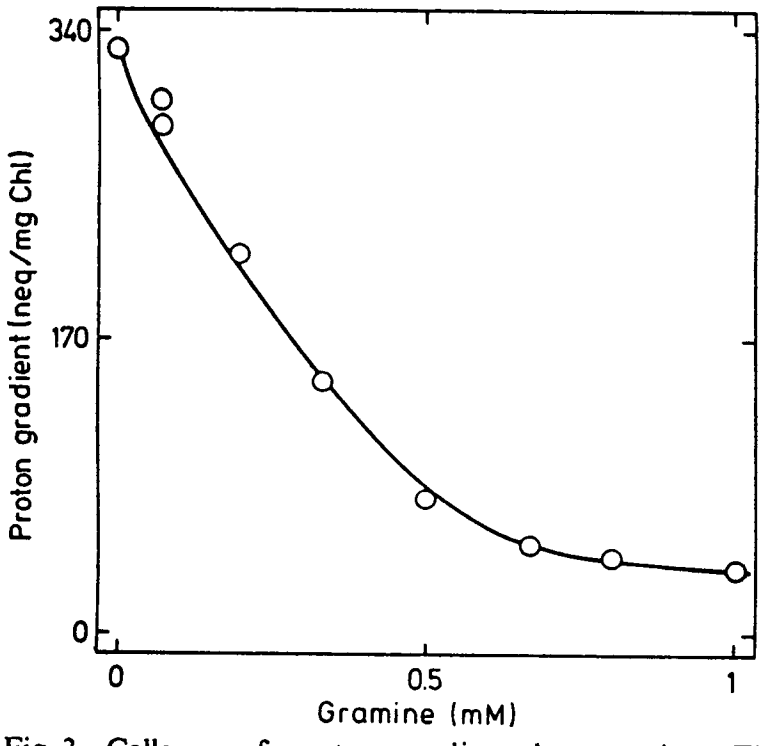

Fig. 3. Collapse of proton gradient by gramine. The pH changes of thylakoid suspensions were measured with a pH-meter equipped with a combination electrode and a recorder. The reaction medium (3 ml) was 10 mM NaCl, 50 μM phenazine methosulfate, and thylakoids 150 μg of chlorophyll, prepared as usual but washed and resuspended in 10 mM NaCl.

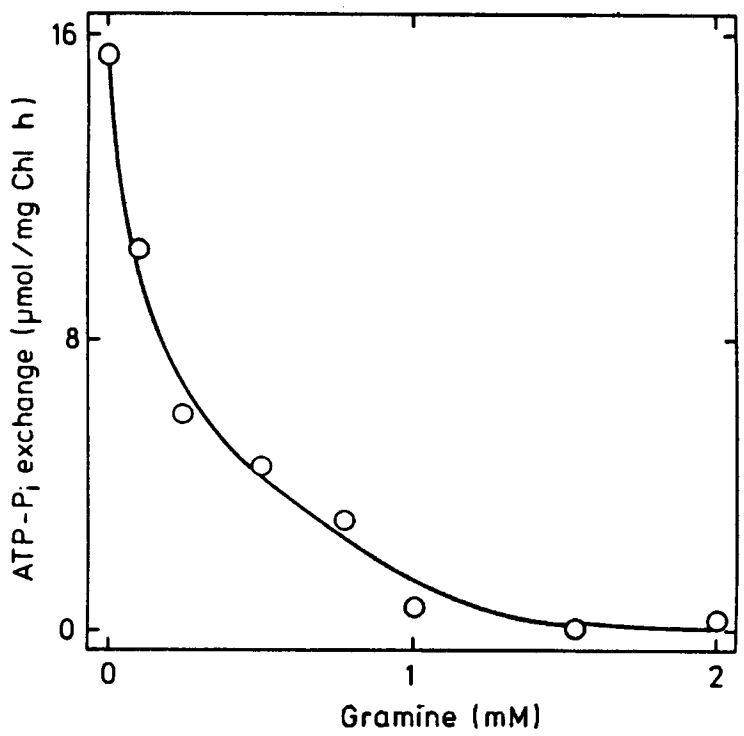

Fig. 4. Inhibition of the Pi-ATP exchange reaction by gramine. Experimental conditions were as described in the text.

at high rates. In contrast, direct inhibitors of photophosphorylation, block both phosphorylation and that portion of electron transport that is a consequence of proton efflux linked to phosphorylation (see ref. 12 for a discussion of inhibition of photophosphorylation). Thus, the described inhibition of photophosphorylation produced by gramine can be explained by an effect of the alkaloid on either the electron transport or the energy transfer reactions.

In order to obtain further information we studied the effect of gramine on the photosynthetic electron transport and on the light-dependent proton gradient. Figure 2 shows that electron transport from water to methylviologen in both basal and coupled conditions were stimulated by addition of gramine at concentrations similar to those affecting ATP synthesis.

Figure 3 shows that the proton gradient, generated by electron transport, was collapsed by addition of gramine. Moreover, the Pi-ATP exchange reaction, which depends on the energization of the thylakoid membrane [10], was also inhibited by the alkaloid at similar concentrations (Fig. 4). These results show that gramine behaves as a typical uncoupler of photophosphorylation [12]. In addition, gramine did not affect the ATPase activity of soluble coupling factor 1 (not shown).

These data give support to the proposal that part of the effects of gramine on mitochondrial activities are due to the abolishment by the alkaloid of the ΔpH across the mitochondrial membrane [6].

It has been postulated that alkaloids, secondary products of plant metabolism, may act as allelochemical agents playing a role in the interaction between different plant species or in the mechanism of defense against plant pathogens or predators [13, 14]. The biochemical basis of this action is, with few exceptions, not well known. The sensitivity of photosynthetic energy conservation machinery to gramine reported in this work may be related to its still unknown biological role in the plants that produce it.

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