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MELITTIN : AN INHIBITOR OF CHLOROPLAST PHOTOCHEMICAL REACTIONS

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SUMMARY. Melittin, a polypeptide component of bee venom, is an inhibitor of photochemical reactions in chloroplasts isolated from higher plants. At concentrations around 5 μM, melittin acts as an uncoupler of photophosphorylation and abolishes the 518 nm light induced absorbance changes. At higher concentrations (30-50 μM), melittin abolishes both the light-induced photooxidation of cytochrome f, and partially inhibits other reactions of photosynthetic electron transfer, without causing lysis of the membrane. The observed inhibitions appear to be due to changes in the properties of the membrane lipid bilayer, caused by penetration of melittin molecules.

The polypeptide melittin, a major component of bee venom, is comprised of twenty-six amino acids, of which the twenty N-terminal residues are hydrophobic and the six C-terminal residues are basic (1). Although melittin itself is devoid of enzymic activity, it potentiates the activity of bee venom phospholipase, and causes lysis of membranes and phospholipid liposomes (2). This communication reports the ability of melittin to act as an inhibitor of light-induced, membrane-associated reactions in chloroplasts, without causing lysis of the membrane.

Abbreviations. Asc, ascorbic acid; DCIP, 2,6-dichlorophenolindophenol; DMQ, 2,5-dimethyl-p-benzoquinone; FeCN, potassium ferricyanide; MeV, methyl viologen.

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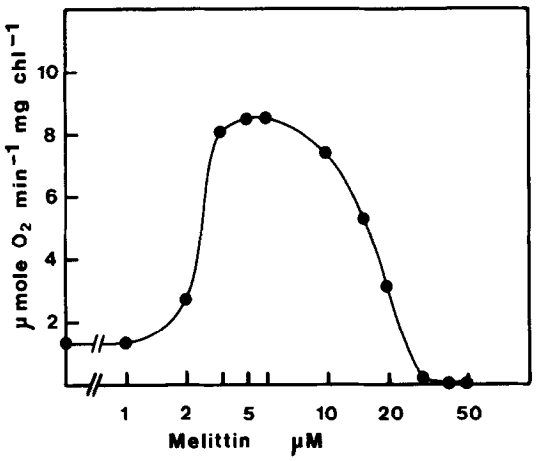

Fig. 1. Effect of melittin concentration on the photoreduction of methyl viologen from water by pea chloroplasts. Note the log scale on the abscissa.

MATERIALS AND METHODS

The techniques for isolation of chloroplasts from peas (Pisum sativum), silver beet (Beta vulgaris), maize (Zea mays) and wheat (Triticum aestivum) and the measurement of the light-induced absorbance change at 518 nm have been previously described (3). The photo-oxidation of cytochrome f was measured as previously described except that far-red light of intensity 1.05 x 10^4 erg cm^{-2} s^{-1} was obtained by the use of a Schott RG 715 filter (3). Photosynthetic electron transfer was measured as described (3) except that methylamine was omitted from the assay, and each incubation contained chloroplasts equivalent to 100 µg chlorophyll. Reaction mixtures were incubated in the dark for one minute before illumination. Melittin (Sigma) was used without further purification or purified by butanol extraction (4) or gel filtration (5).

RESULTS

The effect of increasing concentrations of melittin on the photoreduction of MeV from water is shown in Figure 1. At concentrations below 1 µM, melittin has no effect on the reaction, but as its concentration is increased, the rate of MeV reduction increases until at 5-10 µM melittin, the rate is equivalent to or greater than that obtained by using gramicidin D (2.5 µg/ml) as

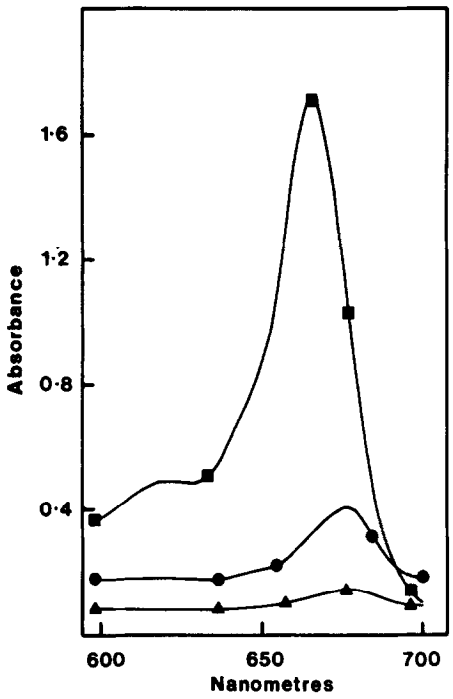

Fig. 2. Release of chlorophyll from pea chloroplasts by treatment with melittin or Triton X-100. After measurement of photochemical activity, the reaction mixture was centrifuged at 10,000 g for 60 sec (Beckman Microfuge) and the absorption spectrum of the supernatant measured. ●, Control; ▲, Melittin, 50 μM; ■, Triton X-100, 0.2%.

uncoupler. Further increases in melittin concentration lead to a progressive inhibition of MeV photoreduction, until at a melittin concentration of 50 μM, the inhibition is complete.

Low concentrations of melittin cause disruption of biological membranes (6,7) and phospholipid liposomes (8). However, concentrations of the polypeptide which completely inhibit photoreduction of MeV from H_2O, cause less release of chlorophyll from the membrane than occurs in the control sample (Fig. 2). Even at a melittin concentration of 100 μM, no solubilization of chlorophyll

TABLE 1

INHIBITION OF PHOTOCHEMICAL ACTIVITY BY MELITTIN

	ACTIVITY μmoles O_2 min^{-1} mg $chlorophyll^{-1}$			
	Control	Melittin 5 μM	Melittin 30 μM	Melittin 30 μM + Plastocyanin 20 μM
H_2O → MeV	1.38	7.21	0.21	4.78
DCIP/ASC → MeV	2.12	7.21	0.95	7.63
H_2O → FeCN	1.48	6.36	3.39	-
H_2O → DMQ	4.03	6.78	5.94	-

occurs. In contrast, Triton X-100 (0.2%) completely solubilizes the chloroplast membrane (Fig. 2) while causing an 88% inhibition of MeV photoreduction. Microscopic examination showed that melittin did not cause significant aggregation of chloroplasts over a two hour period.

The effect of melittin on a number of partial reactions of photosynthetic electron transfer is shown in Table 1. The control value represents the coupled rate. The uncoupled photoreduction of MeV from H_2O (measured in the presence of 5 μM melittin), which is an index of photosystem 1 + 2 activity, is >95% inhibited by 30 μM melittin, while photosystem 1 activity (DCIP/ASC → MeV) is inhibited over 85%. However the inhibition of both these reactions can be relieved by addition of carrier amounts of plastocyanin. Photoreduction of FeCN from H_2O is only about 45% inhibited by 30 μM melittin but this substrate is capable of accepting electrons directly

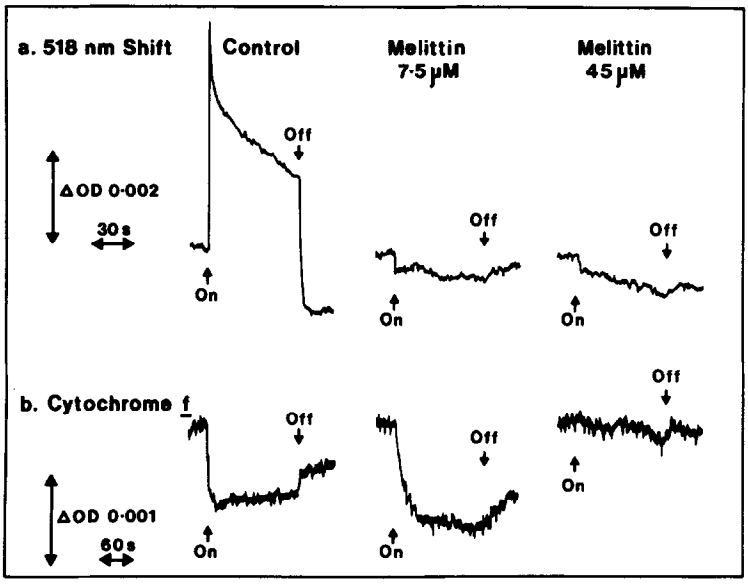

Fig. 3. Effect of melittin on (a) the light induced absorbance changes at 518 nm and (b) the photo-oxidation of cytochrome f by far red light, in pea chloroplasts.

from photosystem 2 as well as from the coupled photo-systems (9). In contrast photoreduction of DMQ, which accepts electrons primarily from photosystem 2 (10) is only 12% inhibited by 30 µM melittin. These results would strongly suggest that inhibition of electron transfer occurs at the plastocyanin site in the chain.

Both the fast and slow components of the light-induced absorbance change at 518 nm, indices of the potential of the chloroplast membrane to generate an electrochemical and proton gradient (11) are abolished by low concentrations of melittin, which uncouple phosphorylation (Fig. 3a). The concentrations of melittin used in this experiment are higher than those used in the other experiments because the incubation mixture contains higher concentrations of chloroplasts,

but the ratio of melittin to chlorophyll remains the same. However, the photo-oxidation of cytochrome f by far-red light (Fig. 3b) is only inhibited at higher concentrations of melittin, similar to those which inhibit MeV reduction, and is not affected by concentrations of the polypeptide which uncouple phosphorylation (Fig. 1) and abolish the 518 nm absorbance changes (Fig. 3a). The inhibition of cytochrome f photo-oxidation by melittin was overcome by carrier amounts of plastocyanin (tracing not shown) in a manner identical to that previously demonstrated for amphotericin B inhibition (3).

Although the data presented have been obtained using pea chloroplasts, similar results have been obtained with maize mesophyll, silver beet and wheat chloroplasts. In all cases the chloroplasts were uncoupled by melittin at low concentrations (5-10 μM), while photoreduction of MeV from water was completely inhibited by melittin concentrations of 50 μM and higher. Purification of melittin to remove any residual phospholipase activity did not affect the inhibition, while bee venom phospholipase A (3 units) did not inhibit the photoreduction of MeV from water, during the period necessary for measurement of photochemical activity.

DISCUSSION

Although melittin is devoid of enzymic activity, it has been shown to have a powerful disruptive effect on membrane systems, due apparently to its ability to penetrate lipid bilayers. It is capable of penetrating the membrane of *Escherichia coli* and potentiating the

activity of an endogenous membrane bound phospholipase A or added bee venom phospholipase A(4). Melittin causes the release of small molecules from phospholipid liposomes (8) and acts as an uncoupler of phosphorylation in mitochondria (12). However it appears that ionic interactions between charged phospholipids and the peptide are not a major factor in these effects, but rather that hydrophobic interactions between the apolar portion of melittin and the hydrocarbon chains of the lipid molecules play a dominant role (8,13).

The present results show that melittin inhibits a number of membrane-localized photochemical reactions in higher plant chloroplasts. From the conclusions derived from studies of melittin-phospholipid interactions (8,13) it seems probable that melittin acts on the membrane lipid bilayer, rather than at one specific site and that the observed inhibitions are due to a modification of the bilayer properties of the membrane. Chloroplast polar lipids are composed largely of uncharged glycolipids (9) and the effect of melittin is probably due to an interaction with the hydrocarbon chains of these lipids.

The concentration of melittin (5 μM) which causes uncoupling and abolishes the 518 nm absorbance changes represents about one molecule of melittin per fifteen molecules of chloroplast polar lipid, while that which totally inhibits MeV reduction represents about one molecule of melittin per two molecules of polar lipid. We have demonstrated (unpublished observations) that melittin rapidly penetrates monolayers of chloroplast

polar lipids, and it has also been shown by ^{13}C-NMR, that when melittin is incorporated into liposomes of chloroplast lipids, a severe restriction of motion of the carbon atoms in the lipid hydrocarbon chains can be detected (J.M. Coddington, personal communication). We conclude therefore that the observed effects of melittin on chloroplasts are due to its penetration into the membrane lipid bilayer, causing a restriction in motion of the lipid molecules, which leads, at low melittin concentrations, to an inability of the membrane to establish an electrochemical and proton gradient in a manner analogous to that previously demonstrated for amphotericin B. At higher melittin concentrations the mobility of the membrane lipids are further restricted, to the extent that plastocyanin, which is a water soluble protein located near the inner surface of the thylakoid membrane, diffuses from its site, resulting in an inhibition of electron flow.

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