Interference by Herbicides with the Transmembrane Potential of Thylakoids

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Interferences expressed by herbicides classified as inhibitory uncouplers were measured on the induction and maintenance of ΔpH and $\Delta\Psi$, the chemical and electrical components, respectively, of the proton motive force (pmf) generated by light-induced cyclic electron transport in spinach thylakoids. Maintenance of the pmf is required for the synthesis of ATP. The inhibitory uncouplers are known to inhibit photophosphorylation, but the mechanisms involved remain to be identified. The dinoseb types (dinitrophenols, benzimidazoles, benzonitriles, bromophenoxim, perfluidone, thiadiazoles) of inhibitory uncouplers, most of which contain dissociable protons, were found to discharge ΔpH at low concentrations and to collapse $\Delta\Psi$ at high concentrations. Collapse of ΔpH can be attributed to the protonophoric (proton shuttling) action of the herbicides. However, collapse of $\Delta\Psi$ can be caused by alterations induced to the integrity and loss of semipermeability of the thylakoid membrane. As a result the membrane becomes permeable to protons and other cations, and the electrical charges across the membrane are neutralized. The non-ionic dicryl types of inhibitory uncouplers (acylanilides, dinitroanilines, diphenylethers, biscarbamates) collapsed $\Delta\Psi$ at concentrations that were somewhat lower than those required for the collapse of ΔpH. These herbicides appear only to alter the integrity and permeability of the thylakoid membrane. Inhibition of photophosphorylation by the inhibitory uncouplers correlated with their ability to dissipate the pmf.

Introduction

Herbicides that interfere with the light-induced electron transport of isolated thylakoids by interacting with components of the Q_B-protein complex have been divided into two major groups; *i.e.*, electron transport inhibitors and inhibitory uncouplers [1]. In this grouping, the DCMU or diuron-type compounds are classified as electron transport inhibitors and are considered to bind to a 32-kDa protein [2]. Binding is competitive, can be saturated, and is reversible. The association has been designated as high-affinity (specific) binding [3]. The DCMU-type inhibitors are postulated to displace the plastoquinone prosthetic redox group of the binding protein [4]. Herbicides that are considered to act like DCMU include the chlorinated phenylureas, pyridazinones, *s*-triazines, triazinones, uracils, and ureacarbamates [1]. The above herbicides, by inhibiting electron transport, prevent the formation of a proton gradient, or pmf, across the thylakoid membrane required for photophosphorylation.

The inhibitory uncouplers (sometimes referred to as multifunctional inhibitors) inhibit electron transport and also independently act as uncouplers of phosphorylation. Electron transport inhibition results from binding of some of the inhibitory uncouplers to the 32-kDa protein, whereas others (phenolic types) bind to a 41-kDa protein [5]. Binding of the phenolics is non-competitive, does not become saturated, and is irreversible. The association has been designated as medium or low-affinity (non-specific) binding [6]. The inhibitory uncouplers have been divided into two groups: dinoseb (phenolic) types and dicryl (acylanilide) types [7, 8]. Classified as being dinoseb types are phenolics (benzonitriles

Abbreviations: 1. Herbicides: bromofenoxim, 3,5-dibromo-4-hydroxybenzaldehyde-2,4-dinitrophenyloxime; chlorpropham, isopropyl *m*-chlorocarbanilate; dicryl, 3′,4′-dichloro-2-methylacrylanilide; dinoseb, 2,4-dinitro-6-*sec*-butylphenol; diuron (DCMU), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; nitrofen, 2,4-dichlorophenyl-*p*-nitrophenyl ether; oryzalin, 3,5-dinitro-N^4,N^4-dipropylsulfanilamide; perfluidone, 1,1,1-trifluoro-N-[2-methyl-4-(phenylsulfonyl)phenyl]-methanesulfonamide; phenmedipham, methyl *m*-hydroxycarbanilate *m*-methylcarbanilate; propanil, N-(3,4-dichlorophenyl)propionamide; TPU, 1,2,3-thiadiazolyl-3,4-dichlorophenylurea; TTFB, 4,6,7-trichloro-2-(trifluoromethyl)benzimidazole. 2. Others: Chl, chlorophyll; DAD, diaminodurene; DMSO, dimethyl sulfoxide; DPIP, 2,6-dichlorophenolindophenol; FCCP, carbonyl cyanide 4-trifluoromethoxyphenylhydrazone; MV (paraquat), 1,1′-dimethyl-4,4′-bipyridinium; oxonol VI, bis(3-phenyl-5-oxoisoxasol-4-yl)-pentamethine oxonol; pmf, proton motive force; PMS, phenazine methosulfate; PS, photosystem; Q_B, secondary quinone electron acceptor of photosystem II.

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and dinitrophenols), bromofenoxim, and benzimidazoles. The compounds possess dissociable protons and are lipophilic weak acids. Included as dicryl types are various non-ionic acylanilides (propanil, chlorpropham) [1]. Other compounds that can be classified as inhibitory uncouplers, based on literature data, include arylaminotetrahydropyrimidines [9], N-phenyldialkylethaneamines [10], diphenylamines [11], and thiadiazolylanilides [12]. The inhibitory uncouplers in addition to interfering with electron transport also inhibit photophosphorylation by acting as uncouplers in increasing the permeability of the thylakoid membrane to protons. Evidence has been provided that the dinoseb types of herbicides increase proton permeability in two ways [8]. At low concentrations they function as protonophores and at higher concentrations, they alter the integrity of semipermeable membranes so that they become permeable to protons and other cations. The dicryl types only alter the integrity of semipermeable membranes.

Suggestions that the inhibitory uncouplers did more than interfere with electron transport by acting at the Q_B-protein complex are provided by a number of observations including the following: (a) inhibition of phosphorylation at concentrations lower than for inhibition of coupled electron transport [8–15]; (b) inhibition of cyclic phosphorylation [8–10, 12, 15–17]; (c) stimulation of the reduced DPIP $\rightarrow$ MV $\rightarrow$ O_2 reaction conducted under basal conditions [8, 18, 19]; (d) inhibition of the uptake of lipophilic fluorescent amines [8, 14, 19, 20]; (e) inhibition of valinomycin-induced swelling of thylakoids and intact chloroplasts [8, 18, 19]; (f) induction of swelling of thylakoids and intact chloroplasts in the absence of an ionophore [8, 19]; (g) alteration to the permeability of chloroplast membranes to endogenous potassium [19]; (h) alteration to the permeability to protons of artificial, purely lipoidal, liposome membranes [18, 19]; (i) interaction with CF_1 of the ATP coupling factor complex (nitrofen [21, 22], phenols [6]). DCMU-type inhibitors only have limited or marginal effects on the reactions listed above. Some of the above reactions (b, c, and d) are associated with photosystem I, and, hence, involve energization of the membrane. Other reactions (e, f, g, h, and i) do not require light and energization of the membrane is not involved.

The objectives of the study reported herein were to examine the effects of selected herbicides that have been classified as inhibitory uncouplers on the two components that comprise the transmembrane potential, *i.e.*, ΔpH, the chemical component, and $\Delta\Psi$, the electrical component. Effects imposed on the transmembrane potential cannot be measured under noncyclic conditions because inhibitory uncouplers, with the exception of perfluidone, prevent development of the pmf by inhibiting electron transport through photosystem II by interacting with the Q_B-protein complex, *i.e.*, the compounds inhibit whole chain electron transport. However, effects on the pmf can be measured under conditions that promote cyclic electron flow associated with photosystem I. No evidence has been provided for interference by inhibitory uncoupler herbicides with cyclic electron flow. Consequently, effects on photophosphorylation can conceivably be studied without the confounding of effects imposed by the compounds on electron transport.

Materials and Methods

Thylakoids were isolated from freshly harvested growth chamber-grown spinach (*Spinacia oleracea* L.) by the method of Armond *et al.* [23]. Chlorophyll concentrations were estimated by the method of Arnon [24]. Photochemical reactions were conducted at 25 °C with a photon fluence rate of 750 $\mu mol/m^2 \cdot s$ (PAR). Coupled noncyclic electron transport and photophosphorylation with ferricyanide as the electron acceptor were measured as described by Moreland and Boots [15]. Cyclic photophosphorylation was measured by the procedure of Arntzen *et al.* [25] with PMS as the electron mediator. The esterification of ADP was monitored with a pH electrode by the method of Nishimura *et al.* [26]. Proton transport was monitored with a pH electrode, as described by Dilley [27], in the presence of 0.1 mM DAD instead of pyocyanine. Effects imposed on $\Delta\Psi$ were monitored spectrophotometrically with the optical potential probe oxonol VI as absorbance at 603–590 nm with an Aminco DW-2a dual wavelength spectrophotometer in a medium that consisted of 50 mM tricine-NaOH (pH 8.0), 10 mM NaCl, 5 mM $MgCl_2$, 10 μM PMS, 2 μM oxonol VI and 60 μg Chl in a 2-ml volume. Absorbance changes were converted to mV by calibration against K^+-diffusion potentials in the presence of valinomycin, according to the Nernst equation, as described by Schuurmans *et al.* [28].

Stock solutions of the desired concentrations of test chemicals were prepared in acetone except TPU

which was dissolved in DMSO. The final concentration of solvent was held constant at 1% (v/v) in all assays including the controls. Data presented were averaged from determination made with a minimum of three separate isolations and replications. Standard errors of the I_{50} values, where listed, varied from 1 to 20%.

Results

The herbicides included in the present study are listed in Table I together with data obtained for the uncoupler FCCP which was included as a reference standard. Most of the compounds have been identified as being inhibitory uncouplers and subdivided into dinoseb or dicryl types previously [1, 8]. Nitrofen and TPU were added because of identification as possible activity as energy transfer inhibitors [20, 21]. Phenmedipham was included because of results obtained with isolated mitochondria [29]. Some of the I_{50} values shown for inhibition of coupled noncyclic reduction and phosphorylation have been published previously and are referenced accordingly (Table I, Columns 2 and 3). Other values were determined as part of the present study [29]. For the most part, uncoupling activity is reflected in the lower I_{50} values for phosphorylation relative to coupled electron transport. However, for TPU, dicryl, and phenmedipham the two values were equal. Neither FCCP nor perfluidone inhibited electron transport.

All of the compounds including FCCP, unlike DCMU-types of inhibitors, also inhibited cyclic photophosphorylation (Table I). For most compounds, the I_{50} for inhibition of cyclic phosphorylation was slightly higher, *i.e.*, within an order of magnitude, than that for inhibition of noncyclic phosphorylation. However, there are exceptions. For ioxynil, dicryl, propanil, and phenmedipham, the difference is greater than an order of magnitude.

Cyclic phosphorylation depends on the development of a pmf across the thylakoid membrane. Consequently, compounds that inhibit cyclic phosphorylation could prevent or interfere with the development of either ΔpH or $\Delta\Psi$, or both.

Effects induced by compounds on the generation of ΔpH, the chemical component of the transmem-

Table I. Effects of herbicides on photophosphorylation and components of the proton motive force of spinach thylakoids.

Inhibitor	Reduction	Noncyclic[a] Phosphorylation	[Ref.]	Cyclic[b] phosphorylation	Proton[c] transport	Absorbance oxonol VI[d]
	I_{50} [μM]			I_{50} [μM]		
Dinoseb-type						
Bromofenoxim	4.5	2	[8]	6	7	27
Dinoseb	7.5	4	[14]	29	3	138
Ioxynil	0.3	0.2	[14]	86	27	82
Perfluidone	NE[e]	60	[13]	33	3	112
TPU	2	2	[29]	2	1	6
TTFB	4.5	1.3	[8]	4	0.4	10
Dicryl-type						
Chlorpropham	150	140	[14]	120	176	172
Dicryl	3	3	[29]	62	110	86
Nitrofen	22	11	[30]	14	112	14
Oryzalin	> 60	25	[17]	34	45	36
Phenmedipham	2	2	[29]	53	178	62
Propanil	0.7	0.6	[14]	108	210	195
Reference standard						
FCCP	NE	0.35	[14]	1	0.07	1

[a] Ferricyanide was then electron acceptor.
[b] PMS was the electron mediator. Esterification of ADP was monitored with a pH electrode.
[c] Monitored with a pH electrode with DAD as the electron mediator.
[d] Changes in the absorbance of oxonol VI were measured at 603–590 nm.
[e] NE = no effect.

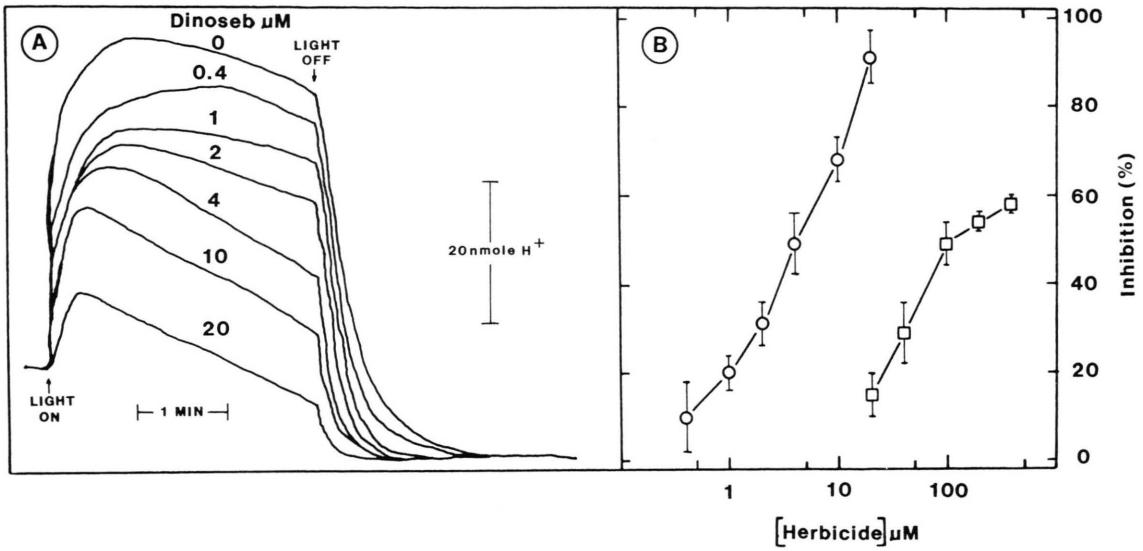

Fig. 1. Effects of dinoseb and dicryl on the light-induced proton gradient formation across spinach thylakoids, mediated by 0.1 mM DAD. (A) Representative traces that show the effects of increasing concentrations of dinoseb on proton transport. (B) Dose-response curves, plotted semi-logarithmically, that show inhibitory effects imposed by dinoseb (○———○) and dicryl (□———□) on proton transport. Data are presented as means ± SD (shown as error bars) for three isolations of thylakoids.

brane gradient can be measured as proton transport with a pH electrode. Shown in Fig. 1 A are representative traces that depict the alkalinization of the external medium caused by H^+-transport into the loculus of spinach thylakoids catalyzed by DAD-mediated cyclic electron flow (no-inhibitor control curve). In the dark, the pH (7.0) of the medium is stable. Illumination caused a rapid increase in the pH of the medium that declined slowly with time after 1 min. The slow decline appeared to be an artifact caused by the shining of light (or the generation of heat) on the pH electrode, *i.e.*, similar changes in pH occurred when thylakoids and DAD were not present in the reaction mixture. When the light was turned off, the protons reequilibrated across the membrane and the pH returned to essentially the initial value. The addition of inhibitory uncoupler herbicides such as dinoseb caused the extent of the pH change to decrease in a concentration-dependent manner as shown in Fig. 1 A. The effects of inhibitors were quantified by determination of the extent of the pH difference between the point at which the actinic light was terminated and the dark level attained after 3 min. Responses were calibrated by titration with an aliquot of standardized NaOH. From the data obtained, dose-response curves were developed as shown in Fig. 1 B for dinoseb and dicryl. The effects of the herbicides, expressed as I_{50} concentrations, are summarized in Table I. Pure electron transport inhibitors, such as diuron and atrazine, had essentially no effect, at concentrations up to 1 mM, on proton uptake.

I_{50} values for inhibition of proton transport for FCCP and the dinoseb types are, except for bromofenoxim and TPU, considerably lower than for inhibition of cyclic phosphorylation. I_{50} values for inhibition of proton transport were also lower than for inhibition of noncyclic phosphorylation except for bromofenoxim and ioxynil. The relation between I_{50} values for inhibition of proton transport and inhibition of cyclic phosphorylation for dicryl types was reversed, *i.e.*, lower I_{50} values were obtained for inhibition of cyclic phosphorylation than for inhibition of proton transport. However, for most of the compounds, the I_{50} values differed only by a factor of two. Compared to inhibition of noncyclic phosphorylation, the I_{50} for inhibition of proton transport approached or was greater than two orders of magnitude for dicryl, propanil, and phenmedipham. For chlorpropham and oryzalin, the comparative values

differed by less than a factor of two, whereas for nitrofen, the difference was approximately an order of magnitude.

The initial increase in the pH of the medium corresponded to an apparent uptake of about 1 μmol H^+/mg Chl by the thylakoids. Because the internal buffering capacity of the thylakoids was not known, ΔpH could not be determined directly. However, measurement of the quenching of atebrin fluorescence in parallel experiments yielded a ΔpH value of 2.9, assuming an internal volume of 10 μl/mg Chl [31].

Inhibition of light-induced proton transport also has been reported for the tertiarybutyl analog of dinoseb [6] and for analogs of TPU [20].

Effects induced by compounds on the generation of ΔΨ, the electrical component of the pmf, can be measured with appropriate anionic dyes such as oxonol VI [28]. The transmembrane electrical potential component (ΔΨ) of the pmf of thylakoids results from the charge separation across the membrane caused by the primary photochemical acts associated with the reaction centers. The potential generated can be monitored by measuring absorbance changes ($\Delta A_{603-590}$) that are associated with the movement of oxonol VI (2 μM) across the membrane. Representative traces of the light-induced oxonol VI absorbance changes under conditions of cyclic electron flow (10 μM PMS) are presented in Fig. 2A. As shown in the no-inhibitor control curve, upon illumination, there is a rapid inward movement of oxonol VI anions as reflected in the initial increase in absorbance. Subsequently, under steady state conditions, proton translocation is considered to cause cation efflux (K^+, Mg^{2+}) and some anion (Cl^-) influx that results in the lowering of ΔΨ to about 40 mV in favor of ΔpH [28, 32]. Consequently, some of the oxonol VI leaves the loculus and the absorbance decreases. When actinic illumination was terminated, the absorbance decreased rapidly as the light-induced charge separation decayed, and ΔΨ dissipated. The level of ΔΨ exhibited a transient drop below the initial dark value because the subsequent reequilibration of the counter ions across the membrane was a slower process than the decay of the light-induced charge separation. Consequently, a small potential of the opposite polarity developed that required about 20 sec to collapse. All of the inhibitory uncouplers decreased the movement of oxonol VI into the loculus. This action is shown in the traces obtained with increasing concentrations of dinoseb (Fig. 2A). The transient decrease in ΔΨ below the initial level,

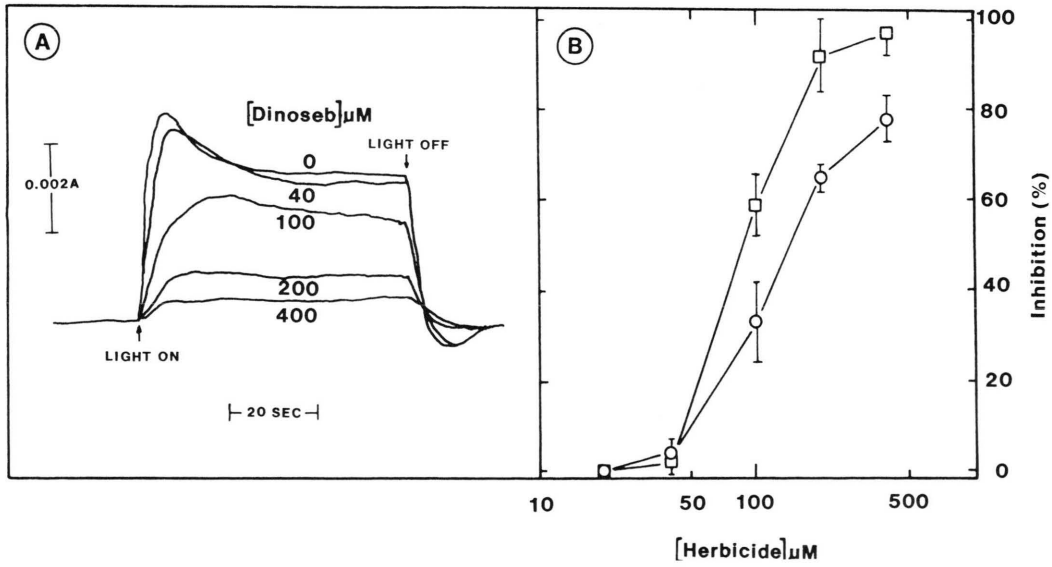

Fig. 2. Effects of dinoseb and dicryl on the light-induced membrane potential (ΔΨ) of spinach thylakoids. (A) Representative traces that show the effects of increasing concentrations of dinoseb on ΔΨ, monitored as absorbance shifts of oxonol VI at 603–590 nm. (B) Dose-response curves, plotted semi-logarithmically, for effects imposed by dinoseb (○———○) and dicryl (□———□) on ΔΨ. Data are presented as means ± SD (shown as error bars) for three isolations of thylakoids.

when illumination was terminated, did not occur at the higher inhibitor concentrations. This reflected a smaller counter ion flux and/or an increased rate of reequilibration in the dark. The effects of inhibitors on $\Delta\Psi$ were quantified by determination of the extent of the absorption difference between the point at which the actinic light was terminated and the dark level attained after 20 sec. Dose-response curves, such as those shown for dinoseb and dicryl in Fig. 2B, were developed from the data obtained.

I_{50} values for all of the compounds were obtained from dose-response curves such as shown in Fig. 2B for dinoseb and dicryl, and are listed in the last column of Table I. I_{50} values for FCCP and the dinoseb types were all higher than for inhibition of proton transport, *i.e.*, the dinoseb-types have a stronger effect on ΔpH than on $\Delta\Psi$. The situation is reversed for the dicryl types where the I_{50} for inhibition of oxonol partitioning was slightly lower than the I_{50} for inhibition of proton transport. However, for some compounds (chlorpropham, oryzalin, propanil) the difference was not statistically significant. Pure electron transport inhibitors, such as diuron and atrazine, had essentially no effect on the generation of $\Delta\Psi$, at concentrations up to 1 mM.

The rate of cyclic phosphorylation is known to increase with increasing light intensity, *i.e.*, the rate of proton transport increases with light intensity. This response is shown in the specific activity trace of Fig. 3 where light intensities were increased above the photon fluence rate used in the previous assays. The I_{50} values for inhibition of cyclic phosphorylation by dinoseb, and related compounds, increased with increasing light intensity, whereas the I_{50} values for inhibition by dicryl types increased only slightly (Fig. 3). Herbicides such as dinoseb are considered to act as protonophores. Their ability to collapse the pmf will be related to the rate at which a given con-

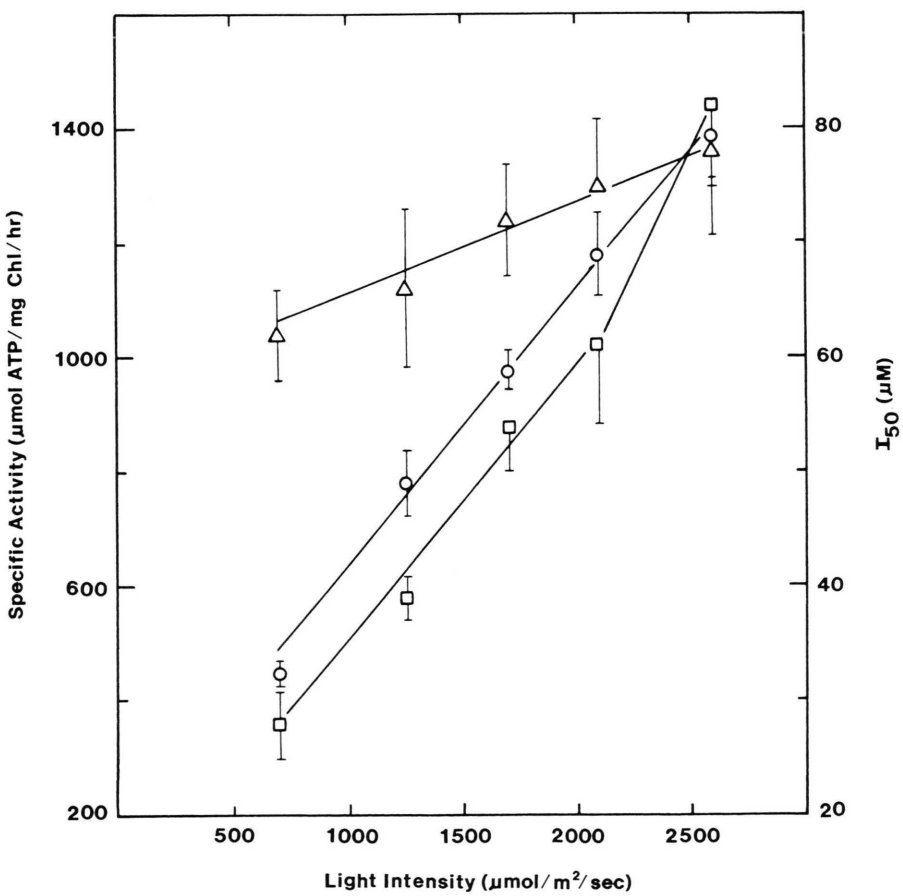

Fig. 3. Effects of light intensity on the specific activity of cyclic photophosphorylation mediated by PMS (○———○), and I_{50} values obtained for inhibition by dinoseb (□———□) and dicryl (△———△), in isolated spinach thylakoids. Data are presented as means ± SD (shown as error bars) for three isolations of thylakoids.

centration of the protonophore can move protons across the membrane in response to the flux of protons in the opposite direction caused by the photochemical act, *i.e.*, an increase in the rate of generation of the pmf will require an increase in the concentration of protonophore required to produce the same amount of collapse of the membrane pmf. As shown in Fig. 3, the I_{50} curve for dinoseb essentially parallels the specific activity curve, as postulated. By contrast, inhibition by dicryl, as reflected in the I_{50} values, did not shift markedly with an increase in proton fluence rates (Fig. 3). The dicryl types of herbicides do not act as protonophores. The extent of the inhibitory response appears to be independent of the magnitude of the proton flux. If the dicryl-type compounds collapse the pmf of the membrane by altering the permeability of the membrane to protons and other cations, as has been postulated [8], the alteration would depend on the concentration of the inhibitor associated with the membrane and not by the magnitude of the proton flux.

Discussion

The consequences of electron transport (both noncyclic and cyclic) is the development of a pmf, or Δp when expressed as mV, across the thylakoid membrane (inside positive). The pmf serves as the primary source of energy for ATP synthesis and other energy coupled reactions. ΔpH is the difference in pH between the outside and inside of the thylakoid membrane. $\Delta\Psi$ is an electrical term that results from the separation of charged ionic species across the thylakoid membrane (inside negative). The major contributor to $\Delta\Psi$ is considered to be the dielectric core of the membrane that is formed by the fatty acid side-chains of the polar lipids and the hydrophobic portions of intrinsic membrane proteins. The electrical potential can arise from diffusion potentials caused by the unequal mobilities or permeabilities of anions and cations across the membrane, and by charge separation across the membrane that results from the primary photochemical events [32]. The electrical and chemical components are interconvertible, hence, Δp tends to remain constant. The magnitude of Δp, and not its individual components, is important relative to its adequacy as a driving force for ATP synthesis.

Considerable attention has been directed towards the quantification of Δp [32, 33]. Most of the techniques used to estimate Δp involve separate determination of $\Delta\Psi$ and ΔpH. The most commonly used techniques are based either upon the steady-state distribution of a permeant ion, assuming electrochemical equilibrium, between the internal and external aqueous phases; altered spectral properties of an optical probe; or involve the use of ion-specific electrodes. $\Delta\Psi$ and ΔpH are calculated from their internal and external concentrations with derivations of the Nernst ($\Delta\Psi$) and Henderson-Hasselbalch (ΔpH) equations, respectively [33].

The quantitative reliabilities of the methods used to measure ΔpH and $\Delta\Psi$ are somewhat arbitrary, are based on assumptions that might not prevail, require estimates of the internal volume of thylakoids that are at best only approximations, have slow response times, and some cannot be calibrated readily [32, 33]. Some of the probes also perturb the membranes and in doing so inhibit electron transport or uncouple phosphorylation. Because of the above limitations and considerations, there are rather large disagreements among results obtained by different investigators using different approaches. Estimates of the values for $\Delta\Psi$ and ΔpH are only approximate and should be considered in a relative rather than an absolute sense.

Literature estimates for Δp, ΔpH, and $\Delta\Psi$ of thylakoids range from 210 to 280, 180 to 240, and 0–100 mV, respectively [32]. Hence, in thylakoids, Δp is almost exclusively ΔpH in the steady state. In the experiments reported herein, ΔpH was estimated at 180 mV and $\Delta\Psi$ at 30 mV, which give a Δp of 210 mV. These values fall within the ranges reported by other investigators.

All of the inhibitory uncouplers affected both ΔpH and $\Delta\Psi$. FCCP and the dinoseb types are considered to act as protonophores, and, hence, discharge ΔpH at low concentrations [8]. At higher concentrations, the compounds affect the physical properties of the membrane by altering their integrity and semipermeability [8]. The alterations are reflected in the collapse of $\Delta\Psi$ caused by the movement of cations in a direction opposite to that of protons, or of anions in the same direction as the protons [32]. An increase in the permeability of the membrane results in an equalization of electrical charges across the membrane, *i.e.*, $\Delta\Psi$ collapses. The dicryl types had a slightly stronger effect on $\Delta\Psi$ than on ΔpH. These compounds are postulated to act primarily by altering the physical properties of the membrane [8]. Such action is reflected in the collapse of both $\Delta\Psi$

and ΔpH which occurs at approximately the same concentration for most dicryl-type compounds. Consequently, the main action of dicryl types involves the equalization of electrical charges across the thylakoid membrane.

Suggestions that the inhibitory uncouplers alter the integrity of the thylakoid membrane also are provided by observations with assays that do not require light and in which electron transport (membrane energization) is not involved. Included are the perturbational and permeability alterations identified and referenced in the Introduction. Loss of membrane integrity also has been postulated, to explain the uncoupling action of arylaminotetrahydropyrimidines [9].

Inhibitory uncouplers interact with the energy transducing mitochondrial inner membrane much like they interfere with the thylakoid membrane. However, in mitochondria, $\Delta\Psi$ comprises approximately 80% of the total pmf and ΔpH the remaining 20%. This is the reverse of the situation in thylakoids. In mitochondria, the dinoseb types affect $\Delta\Psi$ at much lower concentrations than do the dicryl types [29]. Conceivably, the inhibitory uncouplers may perturb all cellular membranes (plasmalemma, tonoplast, nuclear, and endoplasmic reticulum) much like they do to thylakoid and mitochondrial membranes. The perturbations can be expected to alter the many transport, biosynthetic, and regulatory activities associated with membranes. However, the relation between membrane perturbations and the expression of phytotoxicity remains to be identified.

The interaction with membranes may explain the phytotoxicity of some of the herbicides in the absence of light, in nonchlorophyllous tissue, or when PS II has been inhibited. At this time, it is not possible to determine the impact of alterations to the integrity and permeability of organelle membranes on the physiological status of plants, but, small changes, coupled with a reduction in the availability of chloroplast and mitochondrially generated ATP, could have a significant effect over a time span of many hours or several days.

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[1] D. E. Moreland, Annu. Rev. Plant Physiol. **31,** 597–638 (1980).
[2] K. Pfister, K. E. Steinback, G. Gardner, and C. J. Arntzen, Proc. Natl. Acad. Sci. USA **78,** 981–985 (1981).
[3] W. Tischer and H. Strotmann, Biochim. Biophys. Acta **460,** 113–125 (1977).
[4] B. R. Velthuys, FEBS Lett. **126,** 277–281 (1981).
[5] W. Oettmeier, K. Masson, and U. Johanningmeier, FEBS Lett. **118,** 267–270 (1980).
[6] W. Oettmeier, C. Kude, and H.-J. Soll, Pestic. Biochem. Physiol. **27,** 50–60 (1987).
[7] W. R. Alsop and D. E. Moreland, Pestic. Biochem. Physiol. **5,** 163–170 (1975).
[8] D. E. Moreland and W. P. Novitzky, Z. Naturforsch. **39c,** 329–334 (1984).
[9] G. van den Berg, M. Brandse, and J. Tipker, Z. Naturforsch. **37c,** 651–657 (1982).
[10] G. van den Berg and M. Brandse, Z. Naturforsch. **39c,** 107–114 (1984).
[11] W. Oettmeier and G. Renger, Biochim. Biophys. Acta **593,** 113–124 (1980).
[12] G. Schäfer, A. Trebst, and K. H. Büchel, Z. Naturforsch. **30c,** 183–189 (1975).
[13] D. E. Moreland and J. L. Hilton, Herbicides: Physiology, Biochemistry, Ecology (L. J. Audus, ed.), **Vol. 1,** pp. 493–523, Academic Press, London 1976.
[14] D. E. Moreland, S. C. Huber, and W. P. Novitzky, Biochemical Responses Induced by Herbicides (D. E. Moreland, J. B. St. John, and F. D. Hess, eds.), **Vol. 181,** pp. 79–96, ACS Symp. Ser., American Chemical Society, Washington, D.C. 1982.
[15] D. E. Moreland and M. R. Boots, Plant Physiol. **47,** 53–58 (1971).
[16] D. E. Moreland and W. J. Blackmon, Weed Sci. **18,** 419–426 (1970).
[17] D. E. Moreland, F. S. Farmer, and G. G. Hussey, Pestic. Biochem. Physiol. **2,** 342–353 (1972).
[18] D. E. Moreland, Pestic. Biochem. Physiol. **15,** 21–31 (1981).
[19] D. E. Moreland, S. C. Huber, and W. P. Novitzky, Photosynthesis VI, Photosynthesis and Productivity, Photosynthesis and Environment (G. Akoyunoglou, ed.), pp. 521–530, Balaban Int. Science Services, Philadelphia 1981.
[20] G. Hauska, A. Trebst, C. Kötter, and H. Schulz, Z. Naturforsch. **30c,** 505–510 (1975).
[21] B. Huchzermeyer, Z. Naturforsch. **37c,** 787–792 (1982).
[22] B. Huchzermeyer and A. Loehr, Biochim. Biophys. Acta **724,** 224–229 (1983).
[23] P. A. Armond, C. J. Arntzen, J.-M. Briantais, and C. Vernotte, Arch. Biochem. Biophys. **175,** 54–63 (1976).

[24] D. I. Arnon, Plant Physiol. **24,** 1–15 (1949).
[25] C. J. Arntzen, S. V. Falkenthal, and S. Bobick, Plant Physiol. **53,** 304–306 (1974).
[26] M. Nishimura, T. Ito, and B. Chance, Biochim. Biophys. Acta **59,** 177–182 (1962).
[27] R. A. Dilley, Methods in Enzymol. **24,** 68–74 (1972).
[28] J. J. Schuurmans, R. P. Casey, and R. Krayenhof, FEBS Lett. **94,** 405–409 (1978).
[29] D. E. Moreland and W. P. Novitzky, unpublished observations.
[30] D. E. Moreland, W. J. Blackmon, H. G. Todd, and F. S. Farmer, Weed Sci. **18,** 636–642 (1970).
[31] U. Pick and R. E. McCarty, Methods in Enzymol. **69,** 538–546 (1980).
[32] D. R. Ort and B. A. Melandri, Photosynthesis (Govindjee, ed.), **Vol. I,** pp. 537–587, Academic Press, New York 1982.
[33] H. Rottenberg, Methods in Enzymol. **55,** 547–569 (1979).