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Inhibition of Photosynthetic Energy Conversion by Cupric Ion[1]

EVIDENCE FOR Cu^{2+}-COUPLING FACTOR 1 INTERACTION

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ABSTRACT

This study describes a specific Cu^{2+} and light-dependent inhibition of spinach (*Spinacia oleracea* L.) chloroplast reactions involving coupling factor 1 function. A primary effect is an inhibition of photophosphorylation induced by illumination of Class II chloroplasts with micromolar Cu^{2+} and pyocyanine in the absence of ADP, Mg^{2+}, and HPO_4^{2-}. The inhibition, which is dependent on free Cu^{2+} as indicated by protection by ethylene diamine tetraacetic acid and dithiothreitol, requires illumination (electron flow) for establishment of the specific inhibition to be noted. Protection is also afforded by uncouplers and some partial protection is provided by micromolar concentrations of ADP and ATP. The data strongly suggest that Cu^{2+} causes an O_2-independent oxidation of sulfhydryl groups on coupling factor 1, which are essential to catalytic function. This conclusion is supported by the reduction of energy-dependent ^{3}H-*N*-ethylmaleimide labeling of the γ subunit of coupling factor 1 by the Cu^{2+}-light pretreatment.

The experiments of Ryrie and Jagendorf have demonstrated a light-dependent incorporation of nonexchangeable tritium into chloroplast CF_1[3] (21). These findings suggest that a consequence of illumination of chloroplasts is a change in the conformation of CF_1 which may be related to the energy conservation function of chloroplasts and possibly an integral component of the energy-transfer mechanism. McCarty *et al.* have shown that illumination of chloroplasts in the presence of *N*-ethyl maleimide causes an inhibition of photophosphorylation which can be prevented by the presence of ATP or ADP and Pi in the preillumination reaction (15). The inhibition has been shown to be accompanied by an increased reactivity of sulfhydryl groups of the γ subunit of the coupling factor protein to [^{14}C]NEM (16). More recently, other evidence supporting conformational change has been provided by the light-dependent nucleotide responsive inhibition produced by *o*-iodosobenzoate (26) and fluorescence changes in probes bound to CF_1 (13). Additional evidence for nucleotide binding at catalytic sites (6, 20) and on the allosteric sites (4, 7, 9) of grana-bound and soluble CF_1 has been recently reported by several laboratories. Such experiments suggest that nucleotide binding to membrane-bound CF_1 modify its structure and regulate energy-linked changes in conformation which affect its enzymic activity and susceptibility to chemical agents.

Previous reports of Cu^{2+} inhibition of chloroplast reactions have shown that it can inhibit election transport at the oxidizing side of PSII (5) and through a direct inactivation of ferredoxin (22). A preliminary report (25) has delineated the effect of Cu^{2+} on CF_1-dependent reactions of spinach chloroplasts. This communication describes sulfhydryl reactivity and nucleotide-coupling factor interactions which are determinants of a specific energy-linked photoinhibition of phosphorylation by cupric ion.

MATERIALS AND METHODS

Chloroplast Preparation, Preillumination Protocol, and Phosphorylation Assays. Class II spinach (*Spinacia oleracea* L.) chloroplasts were prepared as described previously (12); washed as sedimented pellets with 0.35 M sucrose, 0.05 M NaCl, and 0.01 M Tricine, pH 8.0 (resuspension solution); and then resuspended in this solution at a concentration of 50 or 150 μg of Chl/ml after determination of Chl by Arnon's method (1). Preillumination and phosphorylation were carried out using two different protocols. In protocol A, chloroplasts containing 25 μg of Chl were placed in a standard preincubation mixture containing the following components: 56 mM Na-Tricine, pH 8.0; 17 mM NaCl; 25 μM pyocyanine, in a volume of 1.5 ml; $CuCl_2$, $MgCl_2$, $NaKHPO_4$, and other additions were included in the mixtures as indicated. The 1-min phosphorylation was initiated immediately (no preincubation control) or at the end of a preincubation in light (preilluminated) or darkness (dark controls) by adding 2.5 mM ADP; 5 mM $MgCl_2$, and 5 mM $NaKHPO_4$, and ^{32}Pi containing 5×10^5 cpm in a volume of 0.5 ml to the reactions for cyclic phosphorylation and the same plus 1 mM FeCN to reactions for noncyclic electron flow and coupled phosphorylation to bring the final volume to 2.0 ml. Inhibition by Cu^{2+} is also a function of light intensity and illumination of copper-treated chloroplasts with less than 10^4 ergs cm^{-2} s^{-1} white light does not produce significant inhibition. The inhibition is maximal above 3×10^5 ergs cm^{-2} s^{-1} (data not included). The nature of the light response allowed us to handle control chloroplasts in subdued room light of less than 10^4 ergs cm^{-2} s^{-1} intensity. Preillumination was with heat-filtered white light of intensity 3.5 to 4.0×10^5 ergs cm^{-2} s^{-1}. The completed phosphorylation reaction mixtures were then illuminated for an additional min with light of the same quality and intensity used in the preillumination and then terminated by making to 2% in TCA. ATP synthesis was determined by the method of Avron (2) and ferricyanide reduction as described by Avron, Krogmann, and Jagendorf (3). In other experiments using protocol B, the preincubation stage was identical but containing 75 μg Chl. After preincubation in light or dark, 0.5-ml aliquots were removed to a completed phosphorylation mix of 1.5 ml volume containing ADP, $NaKHPO_4$, and $MgCl_2$ at the concentration shown for protocol A. These protocols are schematically described by Uribe (25).

Uncoupler Protection. Protection by CCCP and NH_4Cl was assessed by a variation of protocol B in which 150 μg of Chl were used in a volume-doubled preillumination reaction mixture. Preincubation in light or darkness was carried out in the presence of 30

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[3] Abbreviations: CF_1, coupling factor 1; NEM, *N*-ethylmaleimide; CCCP, carbonylcyanide, *m*-chlorophenylhydrazone.

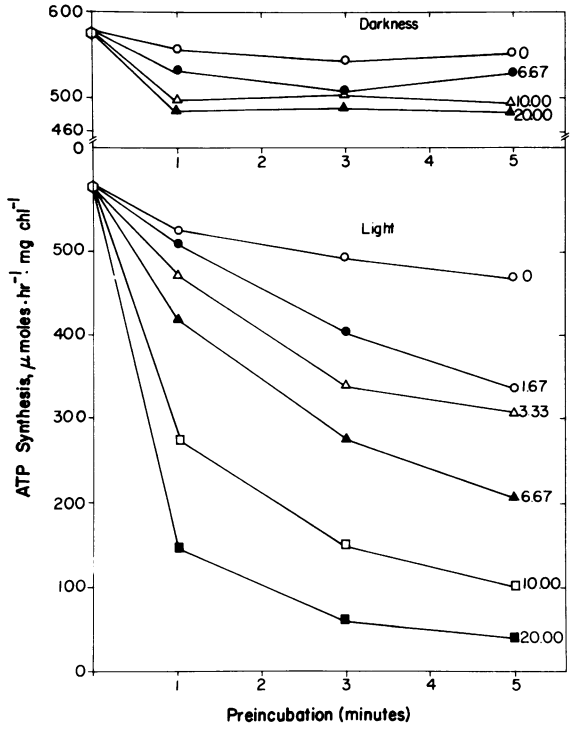

FIG. 1. Time course for photoinhibition. Reactions run at 21°C according to protocol A. Copper chloride, μM concentrations as indicated on figure.

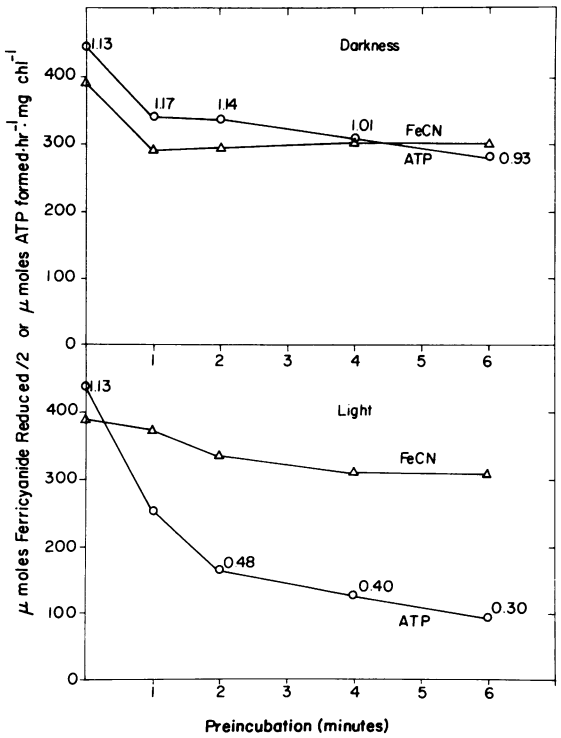

FIG. 2. Effect of Cu^{2+} on noncyclic photophosphorylation. Reactions run at 21°C according to protocol B. Ferricyanide reduction and ATP formation measured as described in "Materials and Methods," with $CuCl_2$ present at 60 μM. ATP/2e values as noted on figure.

μM $CuCl_2$ and 26 μM CCCP or 30 μM $CuCl_2$ and 5 mM NH_4Cl at 0°C. After preincubation, the chloroplasts were recovered by centrifuging at 3000*g* and 4°C for 3 min, and the pelleted chloroplasts were washed with a 10-ml and then a 5-ml aliquot of the

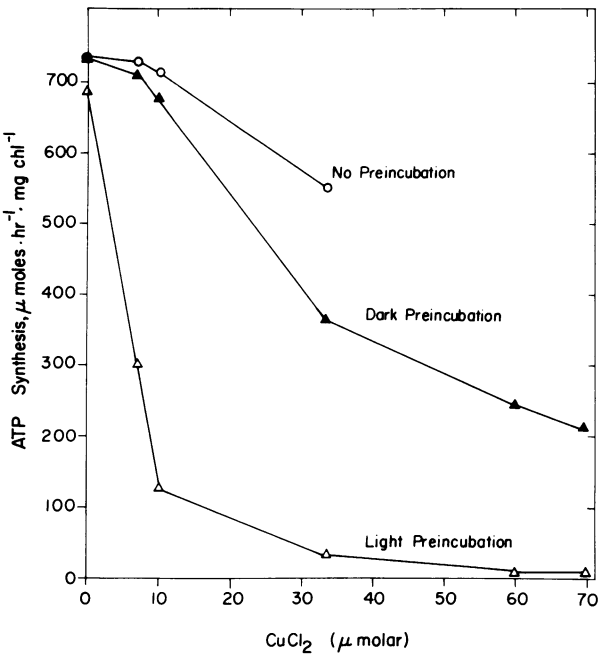

FIG. 3. Inhibition of photophosphorylation by Cu^{2+} in the nonspecific range. Reactions run at 21°C as for Figure 1.

Table I. *Protection by Chelation*

Preincubation was for 5 min using protocol B. Cu^{2+} at 30 μM and EDTA at 0.5 mM where indicated.

Preincubation	Preincubation Conditions	Added After Preincubation	ATP Synthesis
			$\mu mol\ h^{-1}\ mg^{-1}$ Chl
—[a]			620
—[b]			574
+	Light	H_2O	580
+	Light, Cu^{2+}	H_2O	242
+	Light, Cu^{2+}	EDTA	242
+	Light, Cu^{2+}, EDTA	H_2O	584
+	Dark	H_2O	580
+	Dark, Cu^{2+}	EDTA	550

[a] No preincubation control without Cu^{2+}.
[b] No preincubation control plus Cu^{2+}.

resuspension solution. The washed pellets were then resuspended in 1.0 ml of this solution, 2.0 ml of original preincubation solution were added, and 0.5-ml aliquots (25 μg Chl) were removed for assay in the standard protocol B phosphorylation reaction. All experiments included no preincubation controls subjected to the same washing protocol.

ATPase Assays. Trypsin activation of the Ca^{2+} ATPase of membrane-bound CF_1 and soluble CF_1 was carried out using a modification of the method of McCarty and Racker (17). EDTA (5 μmol) and trypsin (50 μg) were added to control or preilluminated chloroplasts. The chloroplasts were then incubated in the dark for 15 min at 32°C. After stopping the proteolysis by addition of excess trypsin inhibitor, the reactions were made 5 mM in $CaCl_2$ and 5 mM in ATP in a final volume of 2.0 ml. Hydrolysis was allowed to proceed for 20 min in the dark at 32°C. The reactions were stopped by the addition of TCA, and the phosphate released was measured by the method of Taussky and Shorr (24). Ca^{2+} ATPase activity of soluble CF_1 was measured in EDTA extracts of dark controls or preilluminated chloroplasts. The pretreated chloroplasts were recovered by centrifugation, and the pellets were

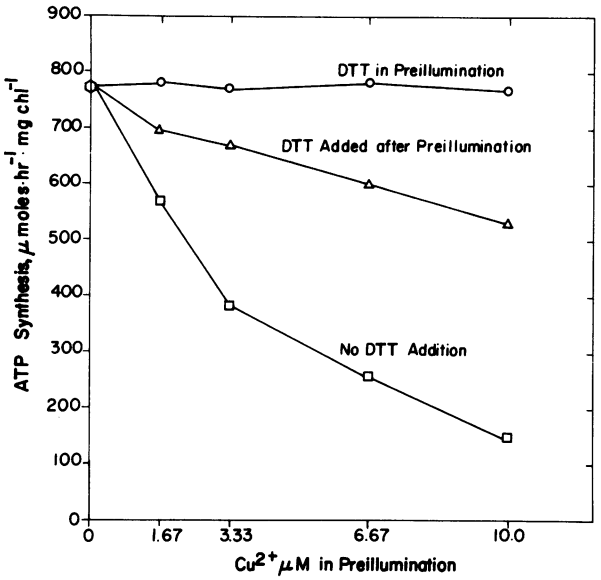

FIG. 4. Effect of DTT on photoinhibition. Reactions run according to protocol A, with a preillumination of 5 min. DTT present at 6.67 mM as indicated.

Table II. *Protection by Uncouplers*

The 4-min preincubation and assay carried out as described in "Materials and Methods."

Preincubation Conditions	ATP Synthesis	+Cu^{2+}, Dark Control
	μmol h^{-1} mg^{-2} Chl	%
Dark, Cu^{2+}, CCCP[a]	3	
Dark, Cu^{2+}, NH_4Cl[a]	27	
Dark, Cu^{2+}	358	
Light, Cu^{2+}	138	39
Dark, Cu^{2+}, NH_4Cl	376	105
Light, Cu^{2+}, NH_4Cl	316	88
Dark, Cu^{2+}, CCCP	305	85
Light, Cu^{2+}, CCCP	232	65

[a] Uncouplers present in phosphorylation stage with NH_4Cl at 5 mM and CCCP at 26 μM.

washed with two 20-ml portions of 10 mM NaCl, then resuspended in 1.5 ml of 1 mM EDTA, pH 8.0, and kept for 15 min at room temperature. The chloroplasts were recovered by centrifugation at 49,000*g* for 25 min, and the supernatant was carefully removed. CF_1 in the EDTA extract was trypsin activated and assayed for Ca^{2+} ATPase as described for bound CF_1.

Proton Pump Activity. Light-induced proton uptake was measured as described previously (11). The preillumination reaction mixture contained 88 mM sucrose; 93 mM NaCl; 0.8 mM Tricine, pH 8.1; 25 μM pyocyanine; and 150 μg of Chl in 4.0 ml. $CuCl_2$ was added at the concentrations indicated. After preincubation at 21°C in light (1.8×10^5 ergs/cm^2·s) or dark for 5 min, the pH was lowered to 6.1 and proton uptake was recorded upon reillumination. The effect of preincubation with Cu^{2+} on phosphorylation by chloroplasts so treated was determined by the method of Nishimura *et al.* (19) after adding 0.23 mM ADP, 0.55 mM $NaKHPO_4$, pH 8.1, and 1.09 mM $MgCl_2$.

NEM Inhibition and Labeling. In experiments on NEM inhibition and labeling, freshly prepared NEM was included in the preincubation mixes at the concentrations indicated. In [^{3}H]NEM labeling experiments, CF_1 was partially purified from the labeled chloroplasts by the chloroform extraction method of Younis *et al.* (27). Two equal portions of each CF_1 sample were then run on SDS-polyacrylamide gels as described by Fairbanks *et al.* (8) (using bromophenol blue instead of pyronin Y as tracking dye), one gel of each pair was stained according to Fairbanks *et al.*, and the other was sliced into fractions using a Bio-Rad 95 gel slicer. The gel slices were incubated for 14 h at 48°C in 0.7 ml of 9:1 (v/v) tissue solubilizer (Baker Scintrex LSC Tissue Solubilizer QT):distilled H_2O mixture. Ten ml of scintillation fluid (5.0 g PPO, 0.10 g POPOP/L toluene) were then added to each fraction;

Table III. *Effect of Nucleotides on Photoinhibition*

Reactions preincubated for 4 min using protocol b. Preillumination stage contained 40 μM $CuCl_2$ and nucleotides and substrates at indicated concentrations. No preincubation control reaction rates plus Cu^{2+} in μmol ATP h^{-1} mg^{-1} Chl were for experiment 1, 543; experiment 2, 519; experiment 3, 458; experiment 4, 439; experiment 5, 469.

	Additions to Preillumination	ATP Synthesis	Inhibition
		μmol h^{-1} mg^{-1} Chl	%
Experiment 1	None	249	54
	ADP, 6.7 μM	305	44
	ADP, 13.3 μM	357	34
	ADP, 27 μM	385	29
Experiment 2	None	192	63
	ADP, 27 μM	313	40
	ADP, 27 μM; Pi, 67 μM	304	41
	ADP, 27 μM; Mg^{2+}, 67 μM	306	41
Experiment 3	None	159	65
	ADP, 27 μM	282	38
	ADP, 27 μM; ASO_4, 67 μM	263	43
	ADP, 27 μM; ASO_4, 67 μM; Mg^{2+}, 670 μM	278	39
Experiment 4	None	179	59
	ATP, 6.7 μM	226	48
	ATP, 27 μM	271	38
Experiment 5	None	210	55
	GDP, 162 μM	240	49
	GDP, 162 μM, Pi, 670 μM	229	51
	GDP, 162 μM; Mg^{2+}, 670 μM	227	52

Table IV. *Effect of Cu^{2+} and NEM on Photoinhibition*

Chloroplasts were preincubated at 21°C with Cu^{2+} for 4 min on NEM for 1 min in single exposures to reagents. In double exposures, chloroplasts were exposed sequentially to Cu^{2+} and NEM for the same times. Copper chloride was present at 30 μM and NEM at 1.0 mM.

Preincubation Conditions	ATP Synthesis	Inhibition
	μmol h^{-1} mg^{-1} Chl	%
No preincubation	550	
Light, Cu^{2+}	238	57
Dark, Cu^{2+}	466	15
Light, NEM	260	53
Dark, NEM	428	22
Light, Cu^{2+} (NEM)	148	73
Dark, Cu^{2+} (NEM)	391	29
Light, NEM (Cu^{2+})	129	77
Dark, NEM (Cu^{2+})	403	27

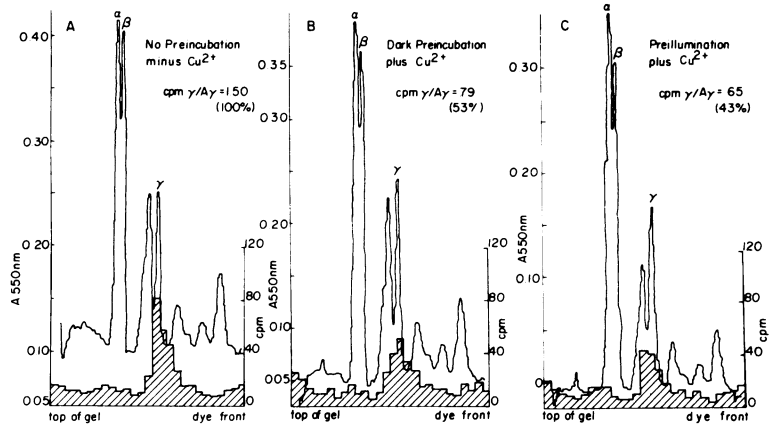

FIG. 5. Diminution of [^{3}H]NEM labeling of CF_1 γ subunit by preillumination of chloroplasts with Cu^{2+}. Two equal portions of each CF_1 sample (pretreated, labeled with [^{3}H]NEM and then partially purified from chloroplasts as described in "Materials and Methods") were run on adjacent SDS-polyacrylamide gels and radioassayed as described in "Materials and Methods" A, No pretreatment, no Cu^{2+} 2-min illumination with [^{3}H]NEM; B, 4-min dark preincubation, 60 μM Cu^{2+} 2-min dark with [^{3}H]NEM; C, 4-min preillumination, 60 μM Cu^{2+} 2-min illumination with [^{3}H]NEM. (Solid line), densitometric tracings of stained gel of each pair; (bar graph), radioactivity (minus blank values) of fractions from sliced gel of each pair. The ratio between the area under the cpm and stained γ subunit peaks (normalized to 100% for panel A) is given in each panel.

the fractions were placed in the dark for 5 h (to minimize chemiluminescence) and then counted in a Packard Tri-Carb 2425 scintillation counter.

Reagents. Pyocyanine was prepared as described by McIllwain (18) and Tricine by the method of Good (10). Trypsin inhibitor, trypsin, ATP, GTP, AMP, and GDP were obtained from Sigma Chemical Company. Water was purified by glass distillation followed by exhaustive ion exchange to a resistance of 18×10^6 ohms. All other reagents were of the purest available commercial grades.

RESULTS

Time and Cu^{2+} Concentration Dependence of Inhibition of Phosphorylation. Preillumination of chloroplasts with cupric ion as described in "Materials and Methods" leads to a time- and Cu^{2+} concentration-dependent inhibition of coupled phosphorylation (Figs. 1 and 2). Both cyclic and noncyclic phosphorylation are strongly inhibited at low Cu^{2+} levels with a maximal extent of inhibition attained within 3 min in the light-dependent range. Lower concentrations of Cu^{2+} produce a lower maximal inhibition and a slower onset. As concentration is increased beyond 20 μM, the inhibition begins to lose light dependence and also becomes nonspecific (Fig. 3). Inhibition of ATP synthesis is accompanied by a slight decrease in electron flow rate (Fig. 2) which thereafter remains stable. The ATP/2e ratio for the noncyclic reaction thus declines as ATP synthesis is progressively inhibited. Inhibition is dependent on electron flow as the presence of an electron carrier such as ferricyanide, pyocyanine, or *N*-methyl phenazonium methosulfate in the preillumination stage is required to achieve a maximal photoinhibition. The inhibition, once established, is stable for at least 60 min under oxidizing conditions in the dark (E. G. Uribe, unpublished).

Requirement for Free Divalent Copper Ion and Oxidizing Conditions. Onset of photoinhibition is dependent on the presence of free Cu^{2+} during the preillumination phase. Inhibition was found to be prevented by EDTA added prior to preillumination (Table 1). The inhibition is stable once established and is then not affected by a subsequent addition of EDTA. Photoinhibition is specific for Cu^{2+} as the chloride salts of Cu^+, Mg^{2+}, Ca^+, Mn^{2+}, Zn^{2+}, and Fe^{2+} were without effect in causing photoinhibition. The effect is not dependent on an autoxidation as it is established to the same level in air or under a N_2 atmosphere (data not included). Reducing conditions (+DTT) during the preillumination are effective in preventing the establishment of photoinhibition. The effect is presumably due to the reduction of Cu^{2+}. The inhibition, once established is reversible to approximately 60% by the addition of DTT (Fig. 4).

Requirement for Energy Input. The requirement for electron flow suggests that the onset of Cu^{2+}-induced photoinhibition is an energy-dependent process. The inclusion of totally uncoupling concentrations of CCCP or NH_4^+ in the preillumination stage of the reaction results in a substantial protection from photoinactivation (Table II). Energy transfer inhibition with either Dio-9 (an experimental antibiotic) or phlorizin, however, does not prevent photoinactivation (data not shown).

Protection by ADP and Other Nucleotides. Energy-dependent alterations in the enzymic function of chloroplast CF_1 have been generally attributed to alterations in the conformation of the coupling factor protein. These changes have been shown to be influenced by the presence of ADP and ATP (15), presumably by binding to allosteric sites on CF_1. Copper-mediated photoinacti-

Table V. *Effect of Preillumination with Cu^{2+} on the Proton Pump and Photophosphorylation*

Preillumination was carried out for 5 min in a 21°C thermostated cell on chloroplasts containing 150 μg Chl. The 4-ml preillumination stage contained: NaCl, 80 mM; pyocyanine, 24 μM; and Cu^{2+} as indicated at pH 8.1. After preincubation, the pH was lowered to 6.1, and proton uptake was measured with a combination electrode. Cyclic phosphorylation was measured by following pH increase at pH 8.1 after addition of ADP, Pi, and Mg^{2+} to 0.27, 0.55, and 1.64 mM, respectively, in a final volume of 5.5 ml. Control activities were: pH rise, 0.67 μeq mg^{-1} Chl and cyclic photophosphorylation, 176 μmol h^{-1} mg^{-1} Chl.

Cu^{2+} in Preincubation	Proton Pump	Photophosphorylation
μM	% inhibition	
11		
Dark	0	17
Light	28	93
22		
Dark	19	48
Light	59	100
88		
Dark	27	100
Light	87	100

vation is also found to be influenced by the presence of specific nucleotides (Table III). Micromolar concentrations of ADP and ATP provide partial protection from photoinactivation with a maximal protection of the order of 25% to 30% at approximately 25 μm for each nucleotide. Magnesium, phosphate, or arsenate added with the nucleotides indicated do not enhance the protective effect. GDP, a phosphate acceptor for the phosphorylation, is not effective in protecting the systems from Cu^{2+}-dependent photoinactivation. Other nucleotides tested including GMP, GTP, and AMP were also ineffective in preventing inhibition. The presence of HPO_4^{2-} and Mg^{2+} also did not enhance the protective effect of any of these nucleotides.

ATPase Activity in Photoinhibited Chloroplasts. Experiments to determine the effect of photoinhibition on the light-activated Mg^{2+} ATPase activity of the chloroplasts are limited by the prevention and reversal of inhibition by DTT. Numerous measurements on the trypsin-activated Ca^{2+} ATPase of chloroplasts photoinhibited with Cu^{2+} with respect to photophosphorylation revealed an ATPase activity equal to that found in controls. The Ca^{2+} ATPase activity CF_1 released by EDTA from preilluminated chloroplasts was also equal to that of the controls (data not shown).

Interaction of NEM with CF_1. A more direct test of interaction of Cu^{2+} with CF_1 is through the application of the covalent labeling agent NEM. The data of Table IV indicate that Cu^{2+} and NEM produce strong inhibition of phosphorylation. When they are applied sequentially under conditions of partial inhibition, an additive reduction in activity is obtained.

The use of [^{14}C]NEM as a covalent labeling reagent, has shown that preillumination in the presence of Cu^{2+} causes a significant reduction in the extent of ^{14}C-labeling of CF_1 (25). The reduction in labeling is associated with the γ subunit of the protein (Fig. 5) shown previously to be specifically labeled by NEM in an energy-dependent reaction (16).

Proton Pump Activity in Photoinhibited Chloroplasts. The data of Table V indicate that the presence of μM Cu^{2+} during the preillumination causes a much more dramatic inhibition of cyclic phosphorylation than of the proton pump. Those concentrations of Cu^{2+} which were found to have a significant effect on proton uptake are well beyond the range of light dependence.

DISCUSSION

Cupric ion, as reported here, is an extremely effective agent for energy-dependent inactivation of phosphorylation. This inhibitory process has similarities to other described inhibitions of phosphorylation and has some important differences as well. Inhibition by Cu^{2+} in the μM range has a rather slow onset time which becomes more rapid as the Cu^{2+} to Chl ratio increases. Energy dependence is also a function of Cu^{2+} concentration. In contrast to other energy-dependent inhibitions reported, the Cu^{2+} effect declines in light dependence as a threshold concentration of Cu^{2+} is reached. This difference is very likely due to the more general reactivity of Cu^{2+} with protein sulfhydryl and amino groups. A general susceptibility of the phosphorylating complex to prolonged exposure of chloroplasts to Cu^{2+} may thus account for the incomplete reversal of inhibition of phosphorylation by DTT (Fig. 4).

The development of maximal inhibition requires high light intensity (3×10^5 ergs/cm^2·s) and inhibits both cyclic (Fig. 1) and noncyclic (Fig. 2) phosphorylation. This energy-dependent inhibition of phosphorylation is characterized by a decline in the ATP/2e ratio from 1.13 to 0.40 with a resultant apparent uncoupling. In contrast, dark preincubation with Cu^{2+} at these same concentrations results in slight inhibition which resembles energy transfer inhibition in that there is a parallel decline in phosphorylation and electron transport (Fig. 2). The rate ferricyanide reduction does not, however, increase in either light or dark preincubation with Cu^{2+}; thus, it is unlikely that the inhibition of phosphorylation is due to uncoupling. In common with other inhibitions of ATP synthesis, the Cu^{2+} inhibition is also moderated by the presence of ADP or ATP in the reaction mix; however, addition of Pi, AsO_4^{2-} or Mg^{2+} with ADP does not enhance the protection afforded. This protection is therefore different from protection from NEM or iodosobenzoate which is provided by GDP, and enhanced by Pi in the presence of ADP (15, 26). Protection that is afforded by the nucleotides is presumably by interaction with allosteric nucleotide binding sites on CF_1 which can regulate energy-linked alternations in the conformation of the protein (7, 23).

The effect of Cu^{2+} on the Mg^{2+} ATPase activity of CF_1 was not testable due to the reversal of inhibition by DTT; however, inhibition of photophosphorylation was not accompanied by a concomitant inhibition of trypsin-activated Ca^{2+} ATPase. This finding was unexpected as those reagents which interact with CF_1 have been found to inhibit both phosphorylation and Mg^{2+} ATPase activity and also the Ca^{2+} ATPase. It is possible that trypsin cleavage of the protein presents an opportunity for regeneration of Cu^{2+}-reacted essential sulfhydryl groups by newly exposed sulfhydryl moieties and could result in a reversal of any preexisting inhibition. Other evidence suggesting that CF_1 is the site of action of Cu^{2+} is the additivity of the Cu^{2+} and NEM inhibition of phosphorylation in the light-dependent concentration range. This is further supported by the reduced labeling by [^{14}C]NEM of CF_1 from light and Cu^{2+}-treated chloroplasts. The reduction in labeling is associated with the γ subunit which has been shown previously to undergo energy-dependent labeling by NEM and iodosobenzoate.

Light-dependent inhibition of phosphorylation by Cu^{2+} is accompanied by an inhibition of the proton pump; however, the two processes are quite differentially affected (Table V). Phosphorylation is much more sensitive showing very strong inhibition after a 4-min illumination with 11 μM Cu^{2+}. Proton uptake is much less susceptible and is not significantly affected at this Cu^{2+} concentration. The proton pump also shows strong sensitivity but only at those concentrations of Cu^{2+} which act independently of light. The light-dependent inhibition of ATP synthesis by Cu^{2+} thus is not due to an inability to generate a proton gradient. Inhibition of the proton pump is thus due to Cu^{2+} binding at sites other than those which affect phosphorylation or at essential sulfhydryl or amino groups of some other less accessible component involved in proton translocation or electron transport.

The interactions of low concentrations of divalent copper ion, with sulfhydryl groups occurs via oxidation, mainly through mercaptide formation which prevents the catalytic function of sulfhydryl moieties. These reaction products are known to be reducible by sulfhydryl reagents which regenerate the native functional group (14). The prevention of inhibition by DTT is likely due to reduction of Cu^{2+} to Cu^{1+}, while the reversal of inhibition is due to reduction to Cu^{1+} and regeneration of essential sulfhydryl groups. At higher Cu^{2+} concentrations beyond the light-dependent range, interaction with protein amino and carboxyl ligands may be additional factors in its effect on photosynthetic energy conservation.

Divalent copper is a potent and, under these experimental conditions, a specific energy-dependent inhibitor of photophosphorylation which reacts with CF_1 presumably through sulfhydryl interaction. The effectiveness of extremely low concentrations of Cu^{2+} suggests that this interaction may be of significance in reducing *in vitro* and *in vivo* photosynthetic energy conversion. Cu^{2+} released from copper proteins or the vacuole during cell fractionation can be effective in this regard. Cu^{2+} introduced as a trace contaminant from distilled or deionized H_2O would also be highly effective. This effect and those described previously (5, 22) may well be of significance in areas of high natural abundance of Cu^{2+} or in areas where it is present as a pollutant.

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