Two Inhibition Targets by $[Cr(2gb)_3]^{3+}$ and $[Co(2gb)_3]^{3+}$ on Redox Enzymes of Spinach Thylakoids†

Agueda E. Ceniceros-Gómez,‡ Beatriz King-Díaz,§ Noráh Barba-Behrens,‡ Blas Lotina-Hennsen,§ and Silvia E. Castillo-Blum*,‡

Departamento de Química Inorgánica and Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F. 04510, México

$[Cr(2gb)_3]Cl(ZnCl_4)$, $[Cr(2gb)_3]Cl_3$, and $[Co(2gb)_3]Cl_3$ were synthesized and characterized. Their chemical structures and the oxidation states of their metal centers remained unchanged in solution. The effects of these compounds, $CrCl_3$ and $[Co(NH_3)_6]Cl_3$, on photosynthesis were investigated. The coordination compounds inhibit ATP synthesis and electron flow (basal, phosphorylating, and uncoupled), behaving as Hill reaction inhibitors. The target for $[Cr(2gb)_3]Cl(ZnCl_4)$ is located at the Q_B level. In contrast, the interaction sites of $[Cr(2gb)_3]Cl_3$ and $[Co(2gb)_3]Cl_3$ are located in the span from P_{680} to Q_A and at the b_6f complex. Neither $CrCl_3$ nor $[Co(NH_3)_6]Cl_3$ inhibited photosynthesis. The 100% inhibition on PS II of $[Cr(2gb)_3]Cl(ZnCl_4)$ is explained in terms of a synergystic effect between the 2gb−chromium(III) coordination compound and the $ZnCl_4^{2-}$ anion.

Keywords: *Chromium(III) complex; cobalt(III) complex; 2-guanidinobenzimidazole; Hill reaction inhibitors; photosystem I and II inhibition*

INTRODUCTION

Co(III) and Cr(III) are two inert metal centers toward substitution processes; therefore, the ligands bound to either of them will remain coordinated. The most common oxidation states for cobalt are II and III. Coordination compounds of cobalt(III) have been used to study electron-transfer reactions with metalloproteins, such as plastocyanin (Sykes, 1985) and ferredoxin (Armstrong, 1982). The oxidized form of these coordination compounds has been made to react with the proteins. On the other hand, the redox inactive chromium(III) complexes inhibit protein oxidation by positively charged coordination compounds. NMR studies employing reduced plastocyanin and chromium(III) coordination compounds have helped in the determination of the binding site of the protein. For these studies, the metalloproteins have been isolated and purified (Cookson et al., 1980a,b; Handford et al., 1980). In the present work we studied the effect of Cr(III) and Co(III) coordination compounds on the thylakoid embedded proteins, expecting to find the same interaction under these conditions.

The effect on photosynthesis of metal salts containing metal ions in oxidation state II such as Cu, Zn, and Cd (Sing and Sing, 1987) has previously been investigated. In our group, we have been interested in studying the effect of transition metal ion coordination compounds on photosynthesis. Recently, we found that quinic acid decouples photophosphorylation from photosynthetic electron flow, whereas Co(II) coordination compounds enhance this activity (Barba-Behrens et al., 1993). We have also reported that nickel(II) salts and their coordination compounds with ethyl 5-methylimidazole-4-carboxylate (emizco) behave as Hill reaction inhibitors. These coordination compounds are more potent inhibitors than the salts. It was found that the $NiCl_2$, $NiBr_2$, and $Ni(NO_3)_2$ target is at the b_6f-PC level. On the other hand, the binding sites for the coordination compounds are located at the Q_B-protein and b_6f level. Therefore, they have a common inhibition site located at b_6f-PC, avoiding the PQH_2 oxidation (King-Díaz et al., 1998).

EXPERIMENTAL PROCEDURES

Synthesis and Characterization of the Coordination Compounds. *$[Cr(2gb)_3]Cl(ZnCl_4)$.* Anhydrous $CrCl_3$ (1 g, 6.3 mmol) was suspended in 30 mL of anhydrous methanol, and then 10 mg of Zn powder was added. 2-Guanidinobenzimidazole (3.316 g, 18.9 mmol) was dissolved in 30 mL of anhydrous methanol. This solution was added to the suspension, and it was vigorously stirred. This mixture was later heated (40 °C) for ∼30 min. A pink solid precipitated. This was filtered off and dried in vacuo.

$[Cr(2gb)_3]Cl_3$. Anhydrous $CrCl_3$ (0.158 g, 1.0 mmol) was placed in the filter thimble of a Soxhlet extractor together with Zn−Hg amalgam (1 cm^3), and a solution of 2-guanidinobenzimidazole (0.5256 g, 3 mmol) in 60 mL of anhydrous methanol was added to the reservoir flask. Oxygen-free dried nitrogen was bubbled through the methanol for 10 min before heating, and the chromium chloride was then extracted as a deep green solution and reacted with the 2-guanidinobenzimidazole for ∼150 min. A pink solid precipitated, which was filtered off and dried in vacuo.

$[Co(2gb)_3]Cl_2NO_3$. $[Co(NH_3)_4(CO_3)]NO_3$ (0.124 g, 0.5 mmol) was suspended in 30 mL of methanol, and 1 mmol of HCl (1 mL of 1 N HCl) was added to help the dissolution process, followed by heating and vigorous stirring. 2-Guanidinobenzimidazole (0.262 g, 1.5 mmol) was dissolved in 30 mL of methanol. The solutions were mixed and then refluxed for 2.5 h. Later, a solid precipitated from the mixture and was dried in vacuo.

$[Co(NH_3)_6]Cl_3$ was synthesized as previously described (Williams, 1989).

The compounds were characterized by elemental analyses, IR, UV−visible absorption spectroscopies, and magnetic sus-

* Author to whom correspondence should be addressed (fax +52 5 622 3724; e-mail blum@servidor.unam.mx).

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‡ Departamento de Química Inorgánica.

§ Departamento de Bioquímica.

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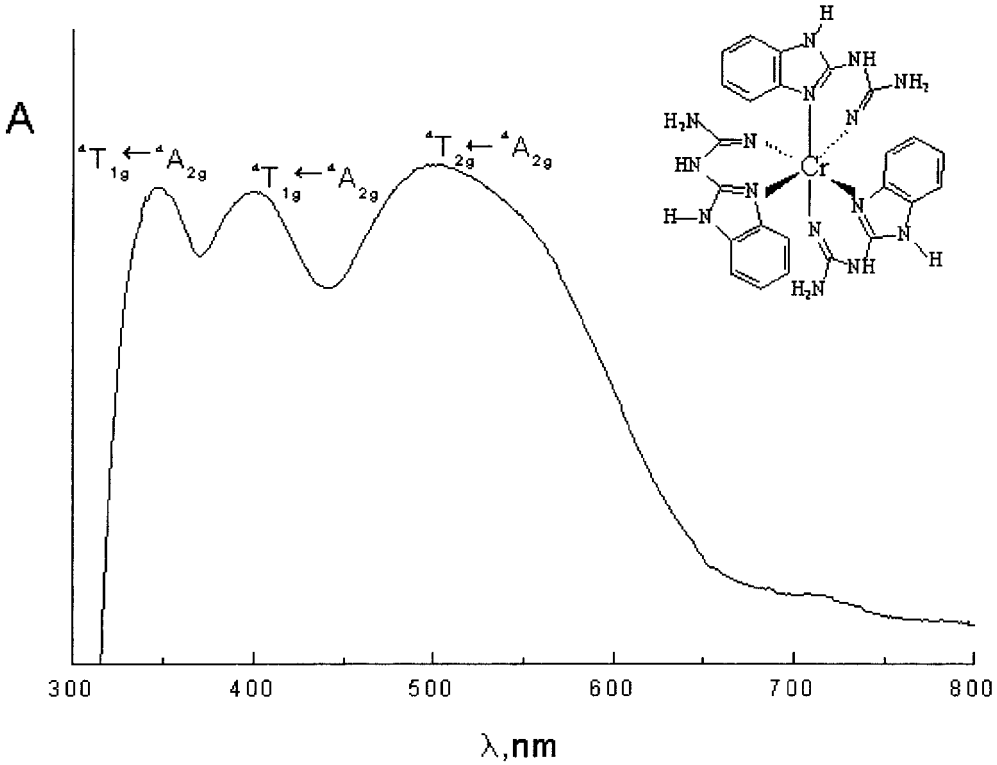

Figure 1. Solid-state UV−visible absorption spectrum of the $[Cr(2gb)_3]^{3+}$ cation.

ceptibility. The cobalt compound was also characterized by ^1H and ^{13}C NMR spectroscopies and X-ray diffraction studies. Figure 1 shows the UV−visible absorption spectrum in solid state (diffuse reflectance) of $[Cr(2gb)_3]Cl(ZnCl_4)$, in which the three expected transitions, in the appropriate energy region, for octahedral Cr(III) are observed, that is, $\nu_1\ ^4T_{2g} \leftarrow {}^4A_{2g}$, $\nu_2\ ^4T_{1g}(F) \leftarrow {}^4A_{2g}$, $\nu_3\ ^4T_{1g}(P) \leftarrow {}^4A_{2g}$ (Lever, 1984). The UV−visible absorption spectra in the solid state were obtained in a Varian Cary 5E spectrometer.

Stability studies were performed in aqueous buffered solutions (phosphates and cacodylate), pH 5.7, 6.3, 7.0, 7.5, and 8.0 $\mu = 0.1$ M (NaCl).

Cyclic voltammetry measurements using microelectrodes of Pt, Au, or glassy C as the working electrode, Pt as the counter electrode, and Ag/AgCl as the reference electrode were carried out. $[Co(2gb)_3]Cl_2NO_3$ (0.18 g) was dissolved in 25 mL of water, or in 25 mL of a 0.1 M MOPS buffer solution (pH 7.0), to give a 1 mM solution in the cobalt complex. These solutions were used for the electrochemical experiments, for which the potential range used was from 2000 to −800 mV and the scan rate from 10 to 200 mV/s. The equipment employed was an EG&G PAR potentiostat/galvanostat Model 273 A.

Thylakoids Isolation and Chlorophyll Determination. Spinach leaves (*Spinacea oleracea* L.) were obtained from local markets. Chloroplasts were isolated from fresh and turgid leaves by grinding the leaves for 5 s in an Osterizer blender in the isolation medium: 400 mM sucrose, 10 mM KCl, 5 mM $MgCl_2$, and 30 mM tricine−KOH (pH 8). The slurry was filtered through eight layers of cheesecloth. Intact chloroplasts were sedimented by centrifugation at 1269*g* for 5 min. They were suspended in the isolation medium and stored as a concentrated suspension in the dark for 1 h at 0 °C (Lotina-Hennsen et al., 1998; Mills et al., 1980; Saha et al., 1971). Measurement of ferricyanide reduction at 420 nm before and after chloroplast lysis (incubated in the lysing medium) indicated ~60% intact chloroplasts in the preparations. Thylakoids were obtained from freshly lysed intact chloroplasts by incubatoin in the lysing medium: 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, and 30 mM tricine−KOH (pH 8), prior to any photosynthetic activity measurements (Mills, 1980). They were immediately used for ATP synthesis and electron flow determinations. Electron microscopy observations showed that all chloroplasts are lysed in the sorbitol-containing incubating medium (data not shown). Chlorophyll was determined according to the method of Strain et al. (1971).

Measurement of ATP Synthesis, H^+ Uptake, and Electron Transport. The light-induced ATP synthesis was determined titrimetrically with a suspension of thylakoids (60 μg of chlorophyll) using an Orion Model 8103 Ross microelectrode connected to a Corning Model 12 potentiometer, with expanded scale as reported (Dilley, 1972; Jiménez et al., 1998). The pH changes between pH 8.0 and 8.1 were registered using a Gilson recorder. The ATP synthesis reaction medium used contained 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM KCN, 50 μM MV, and 1 mM HEPES−KOH (pH 8.0), with added 1 mM ADP and 3 mM phosphate when the intact chloroplasts were freshly lysed.

Proton uptake was measured as a pH rise between pH 8.0 and pH 8.1 as described by Dilley (1972) using the equipment for ATP measurement. The medium used contained 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM KCN, 50 μM MV, and 1 mM HEPES−KOH (pH 8.0), where the intact chloroplasts were freshly lysed.

Photosynthetic noncyclic electron transport in the presence of methylviologen was monitored with a YSI (Yellow Spring Instrument Co.) Model 5300 oxygen monitor using a Clark electrode in a temperature-regulated flask at 20 °C. The reaction medium contained 100 mM sorbitol, 10 mM KCl, 5 mM $MgCl_2$, 50 μM MV, 0.5 mM KCN, and 15 mM HEPES−KOH (pH 8.0); thylakoids at a Chl concentration of 20 μg/mL were added to the reaction medium. The sample was illuminated for 1 min in the presence or absence of 6 mM NH_4Cl (Lotina-Hennsen et al., 1998; Saha et al., 1971).

Uncoupled PS II electron transport was measured by photoreduction of DCPIP-supported O_2 evolution monitored polarographically. The reaction medium for assaying PS II activity contained the same whole-chain electron transport medium ($H_2O \rightarrow$ MV) above-mentioned without methylviologen, but in the presence of 1 μM DBMIB, 100 μM DCPIP, 300 μM $[Fe(CN)_6]^{3-}$, and 6 mM NH_4Cl. Uncoupled photosystem II electron transport from water to DCBQ (Yruela et al., 1991) was measured polarographically with a reaction mixture as in photosystem II, with the addition of 100 μM DCBQ, 1 μM DBMIB, and 6 mM NH_4Cl without DCPIP and $[Fe(CN)_6]^{3-}$.

Uncoupled photosystem II electron transport from water to silicomolybdate was measured with a reaction mixture as in photosystem II except that 200 μM silicomolybdate (electron acceptor), 10 μM DCMU (inhibitor), and 6 mM NH_4Cl were added (Giaquinta and Dilley, 1975). Uncoupled photosystem II electron transport from diphenylcarbazide to DCPIP was measured spectrophotometrically as reported by Vernon and Shaw (1969) using Tris-treated thylakoids. DCPIP photoreduction was followed at 660 nm in 3.0 mL of reaction medium, adding 200 μM DPC. Tris-washed thylakoids were prepared by incubating chloroplasts for 30 min at a concentration to give 300 μg of Ch/mL of 0.8 M Tris buffer (pH 8.0 and 4 °C). Uncoupled photosystem I from PQH_2 to MV was measured polarographically; the medium was the same as that from H_2O to MV, except that 200 μM $TMQH_2$ is added as electron donor and 6 mM NH_4Cl as uncoupler plus 10 μM DCMU as the inhibitor of electron transfer from Q_A to Q_B.

Uncoupled photosystem I electron transport was determined in a similar form to noncyclic electron transport. The following reagents were added: 100 μM DCPIP, 300 μM ascorbate, 10 μM DCMU, and 6 mM NH_4Cl (Allen et al., 1974).

Uncoupled PS I electron transport from TMPD/ASC to MV was measured using KCN-poisoned chloroplasts with 500 μM TMPDred/1000 μM ascorbate as the electron donor to P700 and MV as PS I electron acceptor, as well as 10 μM DCMU, 6 mM NH_4Cl, and 1 μM DBMIB to fully inhibit any electron flow prior to PC. Cyanide-treated chloroplasts were prepared by incubating chloroplasts for 30 min at 0 °C in 30 mM KCN and then centrifuging at 7931*g* for 1 min (Sorvall Super T21) and resuspending in the reaction medium (Ouitrakul et al., 1973). TMPD gives fast electron transport that is not coupled to ATP synthesis, and it is also DBMIB-insensitive because it bypasses the plastoquinone proton shuttle (Allen et al., 1974). Results for all of the experiments are the average of three replicates from different batches of thylakoids.

RESULTS AND DISCUSSION

Characterization of the Coordination Compounds in Solution. [Cr(2gb)$_3$]Cl(ZnCl$_4$), [Cr(2gb)$_3$]Cl$_3$, and [Co(2gb)$_3$]Cl$_3$ were synthesized and characterized, as described under Experimental Procedures. The [Cr(2gb)$_3$]$^{3+}$ and [Co(2gb)$_3$]$^{3+}$ cations are stable in solution; that is, their chemical structures remain unaltered after dissolution in aqueous medium, as shown by their electronic absorption spectra. Their UV−visible absorption spectra in solution remain unaltered for at least 3 months. Cr(III) and Co(III) are inert metal ions toward substitution. The inertness toward substitution processes is predicted by the ligand field theory and can be rationalized in terms of the large stability for their electronic configurations, d^3, t_{2g}^3 (Cr^{III}) and d^6, t_{2g}^6 (Co^{III}), for octahedral geometry (Tobe, 1972). Additionally, the compounds are stabilized by the chelate effect. The metal ion is coordinated to three bidentate ligands. That is, the 2-guanidinobenzimidazole (2gb) ligands are bound to the chromium through the imidazolic nitrogen and one nitrogen atom from the guanidine group; therefore, 2gb acts as a chelate. On the other hand, Cr(III) is redox inert, as a very negative potential is required for its reduction or a very high potential is required for its oxidation. For example, $E°$ for the couple Cr^{3+}/Cr^{2+} is −407 mV, where the species involved are the hexaacua ions (Lide, 1995), whereas, for example, for the tris(phenanthroline) complexes, that is, [Cr(phen)$_3$]$^{3+}$/[Cr(phen)$_3$]$^{2+}$, the redox potential is −260 mV. On the other hand, the oxidation process of Cr^{3+} to Cr(VI) takes place only in a highly acidic solution. Therefore, chromium(III) coordination compounds cannot interfere with the thylakoid electron-transfer reactions (Lloyd et al., 1992).

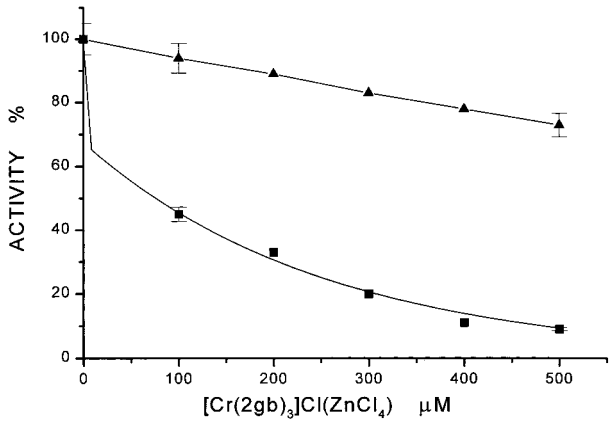

Figure 2. Effect of [Cr(2gb)$_3$]Cl(ZnCl$_4$) on ATP synthesis (■) and H^+ uptake (▲). Results are the average of three replicates from different batches of thylakoids. Rate values for ATP synthesis and H^+ uptake were 1227 ± 17 μmol of $H^+ \cdot h^{-1} \cdot$mg of Chl^{-1} and 125 ± 1.3 μequiv of $H^+ \cdot h^{-1} \cdot$mg of Chl^{-1}, respectively.

[Co(2gb)$_3$]$^{3+}$ could not be reduced to the Co(II) species, as shown by cyclic voltammetry, because this process was outside the electrochemical domain of the water used as solvent (2000 to −800 mV). Therefore, this compound can also be considered as redox inert for the purposes of the experiments described herein.

The $ZnCl_4^{2-}$ anion is a counterion of [Cr(2gb)$_3$]Cl(ZnCl$_4$) that may itself have an important effect on the biochemical properties of the compound. It has been previously shown that Zn(II) salts act as Hill reaction inhibitors. They inhibit both photosystems presenting two targets, one at Q_B and another at b_6f (Miller and Cox, 1983; King-Díaz et al., 1999).

Biochemical Studies. The noncyclic photosynthetic photophosphorylation in spinach thylakoids associated with methylviologen reduction was inhibited by [Cr(2gb)$_3$]Cl(ZnCl$_4$) (Figure 2). The I_{50} (concentration producing 50% inhibition) is 258 μM.

The light-dependent synthesis of ATP by illuminated thylakoids is coupled to the electron flow and may be inhibited by (a) blocking electron flow, (b) uncoupling ATP synthesis from the electron transport, or (c) blocking the photophosphorylation reaction itself.

Thus, the described inhibition of photophosphorylation produced by [Cr(2gb)$_3$]Cl(ZnCl$_4$) can be explained by an effect of the complexes on either the electron transport or the energy-transfer reactions.

To obtain further information, we studied the effect of [Cr(2gb)$_3$]Cl(ZnCl$_4$) on the photosynthetic electron transport and on the light-dependent proton uptake. [Cr(2gb)$_3$]Cl(ZnCl$_4$) inhibited electron transport from water to methylviologen in basal, phosphorylating (in the presence of 1 mM ADP and 3 mM PO_4^{3-}), and uncoupled (6 mM NH_4Cl) conditions (Figure 3), as well as H^+ uptake (Figure 2) at concentrations similar to those affecting ATP synthesis (Figure 2). These results clearly show that [Cr(2gb)$_3$]Cl(ZnCl$_4$) behaves as a typical Hill reaction inhibitor. The I_{50} for uncoupled electron transport is 200 μM.

[Cr(2gb)$_3$]Cl(ZnCl$_4$) completely inhibited uncoupled PS II electron flow from water to DCPIP or DCBQ (Figure 4). The required concentration of this compound for 50% inhibition of PS II was 95 μM (250 μequiv of $e^- \cdot h^{-1} \cdot$mg of Chl^{-1}, as compared with 500 μequiv of $e^- \cdot h^{-1} \cdot$mg of Chl^{-1} for the control).

To localize the inhibition site of [Cr(2gb)$_3$]Cl(ZnCl$_4$) on PS II, electron transport from water to SiMo in the

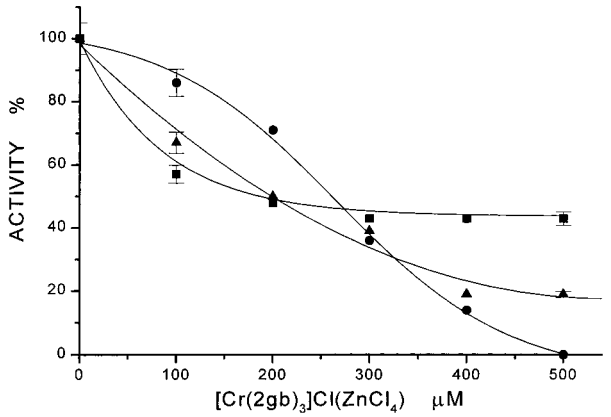

Figure 3. Effect of $[Cr(2gb)_3]Cl(ZnCl_4)$ on basal (■), phosphorylating (●), and uncoupled (▲) electron transport activities. Control = 100% of activity. Results are the average of three replicates from different batches of thylakoids. Rate values for basal, phosphorylating, and uncoupled electron flow were 280 ± 1.7, 620 ± 1.5, and 1197 ± 1.9 μequiv of $e^{-}\cdot h^{-1}\cdot$mg of Chl^{-1}, respectively.

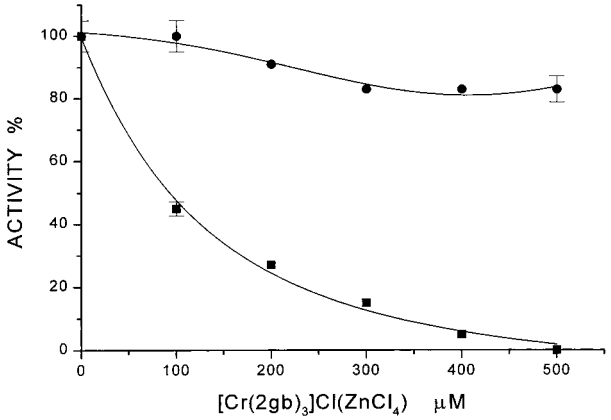

Figure 4. Effect of $[Cr(2gb)_3]Cl(ZnCl_4)$ on PS II partial reactions from H_2O to DCPIP or from H_2O to DCBQ (■) and on PS I partial reaction from $DCPIP_{red}$ to MV (●). Results are the average of three replicates from different batches of thylakoids. Control rate values for PS II and PS I were 667 ± 33, 1750 ± 88, μequiv of $e^{-}\cdot h^{-1}\cdot$mg of Chl^{-1}, respectively.

presence of 10 μM DCMU was measured. Results show that these complexes did not inhibit this span of the electron transport chain, because the electron transport rate for controls was 180 μequiv of $e^{-}\cdot h^{-1}\cdot$mg of Chl^{-1}; this rate was not changed in the presence of 500 μM $[Cr(2gb)_3]Cl(ZnCl_4)$. It is known that SiMo accepts electrons through the Q_A level; therefore, it is concluded that the target for $[Cr(2gb)_3]Cl(ZnCl_4)$ is located at the Q_B-protein or D_1 protein level.

PS I-Supported Electron Flow. To determine the target of $[Cr(2gb)_3]Cl(ZnCl_4)$ beyond Q_B, its effect on uncoupled PS I activity from reduced DCPIP to MV (plus 10 μM DCMU) was determined. PS I electron transport rate was partially inhibited, that is, 30% at 500 μM by $[Cr(2gb)_3]Cl(ZnCl_4)$, suggesting that this compound has a minor inhibition effect on PS I electron transport chain. Therefore, no further studies were performed.

Structure−Activity Relationship Studies of $[Cr(2gb)_3]X$ [X = Cl_3, $Cl(ZnCl_4)$] on Photosynthesis. $[Cr(2gb)_3]Cl_3$ and its salt ($CrCl_3$) were also tested on different photosynthetic activities. The results show that $[Cr(2gb)_3]Cl_3$ also behaves as a Hill reaction inhibitor but with less potency than $[Cr(2gb)_3]Cl(ZnCl_4)$ (Figure 3 and Table 1). For example, the uncoupled electron flow rate from H_2O to MV is 2031 μequiv of $e^{-}\cdot h^{-1}\cdot$mg of Chl^{-1} for the control, which decreases to 563 μequiv of $e^{-}\cdot h^{-1}\cdot$mg of Chl^{-1} in the presence of 500 μM $[Cr(2gb)_3]Cl_3$ (28% remaining activity). In contrast, this activity is 90% inhibited for $[Cr(2gb)_3]Cl(ZnCl_4)$ at 500 μM. It is important to point out that the inhibition site of $[Cr(2gb)_3]Cl_3$ is located from P680 to Q_A, because this compound partially inhibited electron flow on PS II from H_2O to DCPIP, from H_2O to SiMo, and from DPC to DCPIP (explored in Tris-treated chloroplasts; Vernon and Shaw, 1969) (Table 1). On the other hand, $[Cr(2gb)_3]Cl_3$ partially inhibited PS I electron transport from DCPIP to MV (Table 1). It is concluded that its target on PS I is at the level of the b_6f complex because $[Cr(2gb)_3]Cl_3$ does not affect electron flow from P_{700} to MV (Table 1) but inhibits electron transport from $TMQH_2$ to MV. The degree of inhibition is similar to that observed in the electron flow from DCPIP to MV (data not shown). Therefore, $[Cr(2gb)_3]Cl_3$ has two inhibition targets, one located between P_{680} and Q_A, and the second one is at the b_6f complex. The inhibition on the photosynthetic electron transport redox enzyme by $[Cr(2gb)_3]Cl(ZnCl_4)$ is due to the chromium(III) complex cation and to the tetrachlorozincate anion (synergystic effect), because $[Cr(2gb)_3]Cl_3$ acts less efficiently and has another target. Furthermore, the $CrCl_3$ salt is inactive on photosynthetic activities.

Finally, it is shown that the inhibition trends of $[Co(2gb)_3]Cl_3$ and $[Cr(2gb)_3]Cl_3$ on the partial reactions of photosystems II and I are similar, as shown in Tables 1 and 2. Here we are comparing the activities of two 3^+ charged cations; the difference in behavior between the two compounds is due to the fact that one of the coordination compounds contains cobalt, whereas the other has chromium as the central atom.

Both $[Cr(2gb)_3]Cl_3$ (Figure 5) and $[Co(2gb)_3]Cl_3$ inhibited noncyclic photosynthetic photophosphorylation in spinach thylakoids associated with methylviologen reduction. Electron transport from water to methylviologen in basal, coupled, and uncoupled conditions was inhibited by $[Co(2gb)_3]Cl_3$ at concentrations similar to those affecting ATP synthesis (data not shown). These results clearly show that both compounds behave as typical Hill reaction inhibitors.

The inhibition of $[Co(2gb)_3]Cl_3$ on partial reactions on PS I and PS II was similar to that of its chromium(III) analogue, however, with less potency as shown in Table 2. Their effect on partial reactions (photosystems II and I) was investigated using artificial electron donors, electron acceptors, and appropriate inhibitors (Allen et al., 1974). $[Cr(2gb)_3]Cl_3$ and $[Co(2gb)_3]Cl_3$ partially inhibited uncoupled (6 mM NH_4Cl) electron transport in photosystem II from water to DCPIP, from water to SiMo, and from DPC to DCPIP (this last activity was explored in Tris-treated chloroplasts) (Vernon and Shaw, 1969). They also partially inhibited uncoupled photosystem I electron flow from $TMQH_2$ to MV in a percentage similar to that from DCPIP to MV (data not shown) and from $DCPIPH_2$ to MV without effect on uncoupled electron flow from TMPDred to MV (Tables 1 and 2) assayed in KCN-poisoned thylakoids. These results indicate that $[Cr(2gb)_3]Cl_3$ and $[Co(2gb)_3]Cl_3$ primarily inhibited electron flow from P_{680} to Q_A of PS II and partially inhibited at the b_6f complex of PS I electron transport. Therefore, $[Cr(2gb)_3]Cl_3$ and [Co-

Table 1. Effect of $[Cr(2Gb)_3]Cl_3$ on Partial Reactions in Photosystems II and I

	concn (μM)					
	0		200		500	
partial reaction	*a*	*b*	*a*	*b*	*a*	*b*
uncoupled electron transport from H_2O to MV	2031 ± 102	100 ± 5	813 ± 41	40 ± 2	563 ± 28	28 ± 1.4
PS II						
H_2O to DCBQ	667 ± 33	100 ± 5	523 ± 26	79 ± 3.85	333 ± 17	50 ± 2.5
H_2O to SiMo	214 ± 11	100 ± 5	165 ± 8	77 ± 3.8	107 ± 5	50 ± 2.5
DPC to DCPIP	690 ± 35	100 ± 5	83 ± 6.9	12 ± 1	30 ± 1.4	4 ± 0.02
PS I						
$DCPIPH_2$ to MV	1750 ± 88	100 ± 5	1295 ± 65	74 ± 3.7	753 ± 38	43 ± 2.2
$TMPDH_2$ to MV	1700 ± 85	100 ± 5	1790 ± 90	105 ± 5.3	1650 ± 83	97 ± 4.9

[a] Rate of uncoupled electron transport in μequiv of $e^-\cdot h^{-1}\cdot$mg of Chl^{-1}. [b] Percent of activity.

Table 2. Effect of $[Co(2Gb)_3]Cl_3$ on Partial Reactions in Photosystems II and I

	concn (μM)					
	0		200		500	
partial reaction	*a*	*b*	*a*	*b*	*a*	*b*
uncoupled electron transport from H_2O to MV	1666 ± 83	100 ± 5	1222 ± 61	73 ± 3.7	833 ± 42	50 ± 2.5
PS II						
H_2O to DCBQ	667 ± 33	100 ± 5	375 ± 19	56 ± 2.8	208 ± 10	31 ± 1.6
H_2O to SiMo	214 ± 11	100 ± 5	165 ± 8	77 ± 3.9	107 ± 5.3	50 ± 2.5
DPC to DCPIP	690 ± 35	100 ± 5	442 ± 16	64 ± 2.3	283 ± 14	41 ± 2.1
PS I						
$DCPIPH_2$ to MV	1750 ± 88	100 ± 5	1575 ± 79	90 ± 4.5	1179 ± 59	67 ± 3.4
$TMPDH_2$ to MV	1700 ± 85	100 ± 5	1685 ± 85	98 ± 5	1745 ± 88	103 ± 5.2

[a] Rate of uncoupled electron transport in μequiv of $e^-\cdot h^{-1}\cdot$mg of Chl^{-1}. [b] Percent of activity.

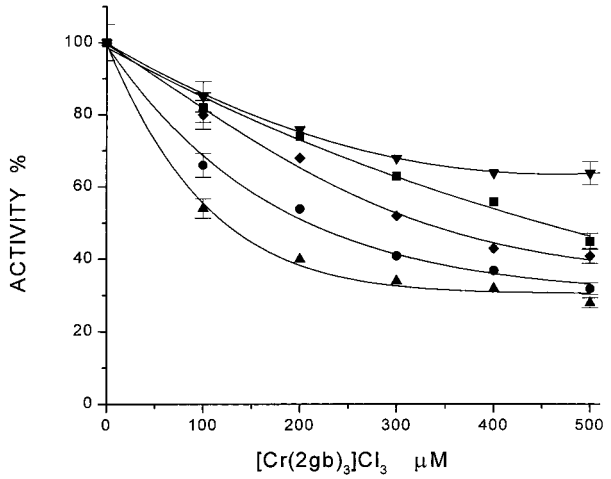

Figure 5. Effect of $[Cr(2gb)_3]Cl_3$ on basal (■), phosphorylating (●), and uncoupled (▲) electron transport rates and ATP synthesis (◆) and H^+ uptake (▼). Results are the average of three replicates from different batches of thylakoids. Control rate values for basal, phosphorylating, and uncoupled electron flow in were 300 ± 3.8, 700 ± 2.2, and 1810 ± 2.1 μequiv of $e^-\cdot h^{-1}$ mg of Chl^{-1}, respectively, whereas rates for ATP synthesis and H^+ uptake were 1200 ± 2.5 μmol of $H^+\cdot h^{-1}\cdot$mg of Chl^{-1} and 129 ± 1.5 μequiv of $H^+\cdot h^{-1}\cdot$mg of Chl^{-1}, respectively.

$(2gb)_3]Cl_3$ have two targets, one at P_{680} to Q_A and the second site at the b_6f-PC complex.

Blank experiments were carried out with the ligand (2gb), a chromium(III) salt, $CrCl_3$, and $[Co(NH_3)_6]Cl_3$, because Co(III) salts are not available. The ligand (2gb) is inactive compared with the control (data not shown), $CrCl_3$ has no effect on any of the photosynthetic activities, and $[Co(NH_3)_6]Cl_3$ acts as a poorly effective uncoupler−Hill reaction inhibitor, without affecting H^+ uptake (Table 3).

Conclusions. The effect on photosynthesis of three isostructural compounds was investigated, namely $[Cr(2gb)_3]Cl(ZnCl_4)$, $[Cr(2gb)_3]Cl_3$, and $[Co(2gb)_3]Cl_3$. All of them behave as Hill reaction inhibitors, $[Cr(2gb)_3]$-$Cl(ZnCl_4)$ being the most potent. Its target on PS II is Q_B. However, the other two coordination compounds have two targets; one is located from P_{680} to Q_A and the other at the b_6f complex. The three compounds have octahedral geometry and a 3^+ charge; the difference in biochemical behavior is attributed to the synergystic effect of the $ZnCl_4^{2-}$ anion in $[Cr(2gb)_3]Cl(ZnCl_4)$ that makes the compound more potent, having at the same time a different target, compared with the other compounds.

Table 3. Effect of $[Co(NH_3)_6]Cl_3$ on Photophosphorylation and Electron Transport Flow

concn (μM)	ATP synthesis[a] (activity %)	basal electron transport[b] (activity %)	phosphorylating electron transport[c] (activity %)	uncoupled electron transport[d] (activity %)
0	100 ± 5	100 ± 5	100 ± 5	100 ± 5
100	115 ± 5.8	109 ± 5.5	114 ± 5.7	100 ± 5
200	105 ± 5.3	127 ± 6.4	129 ± 6.5	100 ± 5
300	90 ± 4.5	146 ± 7.3	143 ± 7.2	100 ± 5
400	85 ± 4.3	146 ± 7.3	150 ± 7.5	100 ± 5
500	85 ± 4.3	146 ± 7.3	157 ± 7.9	100 ± 5

[a] Rate of ATP synthesis = 1633 ± 54 μmol of $H^+\cdot h^{-1}\cdot$mg of Chl^{-1}. [b] Rate of basal electron transport = 423 ± 2.3 μequiv of $e^-\cdot h^{-1}\cdot$mg of Chl^{-1}. [c] Rate of phosphorylating electron transport = 538 ± 3.8 μequiv of $e^-\cdot h^{-1}\cdot$mg of Chl^{-1}. [d] Rate of uncoupled electron transport = 1346 ± 21 μequiv of $e^-\cdot h^{-1}\cdot$mg of Chl^{-1}.

ABBREVIATIONS USED

ASC, sodium ascorbate; b_6f, cytochrome b_6:cytochrome *f* complex; Chl a, chlorophyll *a*; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCPIP, 2,6-dichlorophenol-indophenol; DPC, diphenylcarbazide; DCBQ, dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; *E*°, standard redox potential; 2gb, 2-guanidinobenzimidazole; HEPES, *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid); MV, methylviologen; MOPS, 3-(*N*-morpholino)propanesulfonic acid; phen,

1,10-phenanthroline; PC, plastocyanin; PQ, plastoquinone; PQH_2, reduced plastoquinone; PS II, photosystem II; PS I, photosystem I; Q_A, primary quinone electron acceptor of PS II; Q_A^-, reduced Q_A; Q_B, secondary quinone electron acceptor of PS II; TMPD, tetramethylphenylenediamine; $TMQH_2$, tetramethyl-*p*-benzohydroquinone; tricine, *N*-[tris(hydroxymethyl)methyl]-glycine; Tris, tris(hydroxymethyl)aminomethane; SiMo, silicomolybdic acid hydrate.

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