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Coordination compounds quinic acid as decouplers on photosynthesis

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Abstract

We found that quinic acid (1) inhibits ATP-synthesis and the light dependent H^+-uptake, it also enhances the basal electron transport rate from water to methylviologen in spinach chloroplasts. These results indicate that it behaves as non-classical decoupler on photophosphorylation. The effect on photosynthetic activities of $M(quin)XH_2O$ (M^{2+} = Co, Ni, X^- = Cl; and $M(quin)_2$ (M^{2+} = Co, Zn, Cd) was studied. The compounds $[Co(quin)Cl(H_2O)] \cdot H_2O$ (2), $Co(quin)_2$ (3) $[Ni(quin)Cl(H_2O)] \cdot (H_2O)_2$ (4), $[Zn(quin)_2]$ (5) and $[Cd(quin)_2]$ (6) inhibit ATP-synthesis, dissipate H^+-uptake and accelerate electron transport, behaving as non-classical decouplers. An electronic spectroscopy study of these complexes indicates that they are stable both in water and buffer solutions. The effect of metal salts on photosynthesis was assayed: cobalt chloride has a very mild effect as decoupler, the other metal chlorides (nickel, zinc and cadmium) do not behave as decouplers in freshly lysed chloroplasts.

Keywords: Photosynthesis; Decouplers; Quinic acid complexes; Quinic acid; Non-classical decoupling; ATP synthesis inhibition; Chloroplasts

1. Introduction

Quinic acid (quin) (1) (Fig. 1) is bound to phenols in plants and microorganisms. It can be used as a carbon source when incorporated into the shikimic acid pathway [1]. Phenols have been found to play different functions in plants, one of the most important being the regulation of plant growth [2], but the role of quinic acid in this regulation in unknown. On the other hand, metal ions play a significant role in photosynthesis [3], however, their precise mechanism of action in many processes is not well understood. It has been found that potent decouplers of energy transduction are moderately weak acids or bases and are known to increase proton transfer across membranes, they abolish the link between substrate oxidation and ATP-synthesis without appreciably affecting the electron transfer mediators, although they usually increase the rate of electron transfer. All known decouplers contain:

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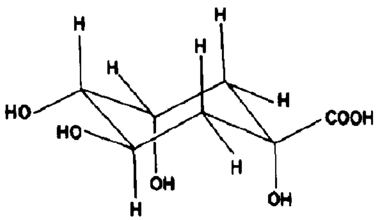

Fig. 1. Structure of quinic acid.

(a) one acid-dissociable group, (b) a strong electron withdrawing moiety, and (c) bulky hydrophobic groups. Most of them are synthetic compounds [4]. However, it was found that some hydrophilic molecules with no proton dissociable groups are very powerful decouplers [5].

We found of interest to study the effect of quinic acid on photosynthesis [6], since it is a natural metabolite, biodegradable, a weak acid and a polyol, and therefore we expected it to behave as a decoupler. Although decouplers have extensively been used as agents for avoiding ATP-synthesis and dissipating a H^+ gradient across membranes, their detailed molecular mechanism of action still remains uncertain. In order to contribute to the understanding of this mechanism, we studied the effect of coordination compounds of quinic acid with bivalent metal ions on photosynthesis. The complexes should have different acid–base, redox and hydrophobic properties from those of quinic acid, therefore we expected that the decoupling properties of the coordination compounds to be different from those of the free acid.

2. Experimental

The metal salts and the solvents were obtained from Merck and J.T. Baker as analytical reagents; quinic acid from Aldrich Chemical Co. The coordination compounds were synthesized as described [7]. Stability of quinic acid coordination compounds was verified by UV–Visible spectroscopy, both in 5 m*M* $MgCl_2$, 100 m*M* KCl, buffered with 1 m*M* Na^+-tricine at pH 8.0 and in water, for 60 min at 25°C. Metal salts were dissolved in the same media and their spectra recorded for 1 h at 25°C. Chloroplast thylakoids were isolated from market spinach leaves (*Spinacea Oleracea L.*) as described earlier [5–9] and suspended, unless indicated, in 400 m*M* sucrose, 5 m*M* $MgCl_2$, 100 m*M* KCl and buffered with 20 m*M* Na^+-tricine at pH 8.0; 50 μM methylviologen was added as electron acceptor for Hill's reaction. Chlorophyll determination [10], photosynthetic non-cyclic electron transport [5–9], H^+-uptake and ATP-synthesis [11] were deter-

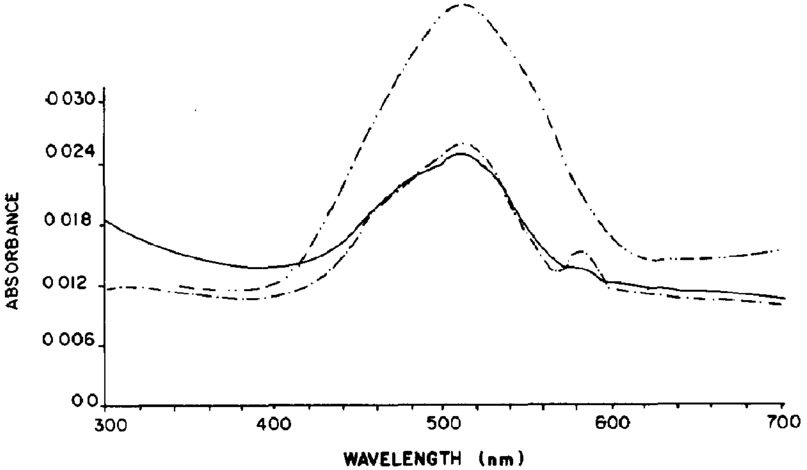

Fig. 2. UV–Vis Spectra of $[Co(quin)Cl(H_2O)]\cdot H_2O$ in (·–·–) 5 m*M* $MgCl_2$, 100 m*M* KCl, buffered with 1 m*M* Na^+-tricine at pH 8.0, ($\varepsilon = 8$ mol^{-1} dm^3 cm^{-1}); (–––) in aqueous solution ($\varepsilon = 8$ mol^{-1} dm^3 cm^{-1}); 25°C. (···–––) and solid state diffuse reflectance.

mined as reported in the literature. All reaction mixtures were illuminated with actinic light of a projector lamp (Gaf 2600) and was passed through a filter of 5 cm of a 1% $CuSO_4$ solution [5–9,11].

2.1. Physical methods

UV–Visible spectra were recorded on a diode array spectrophotometer (Hewlett-Packard) from 190 to 820 nm. The temperature was kept constant (Neslab thermostat) at 25 ± 0.1°C.

3. Results and discussion

The following quinic acid (**1**) coordination compounds were prepared as described [7]: $[Co(quin)Cl(H_2O)] \cdot H_2O$ (**2**), $[Co(quin)_2]$ (**3**), $[Ni(quin)Cl(H_2O)] \cdot 2H_2O$ (**4**), $[Zn(quin)_2]$ (**5**), $[Cd(quin)_2]$ (**6**). Based on their spectroscopic characterization, an octahedral structure was proposed for compounds **2**, **3**, and **4**, and for **5** and **6** a tetrahedral geometry.

We investigated the behaviour of complexes **2** to **6** by UV–Vis spectroscopy in solution (in the

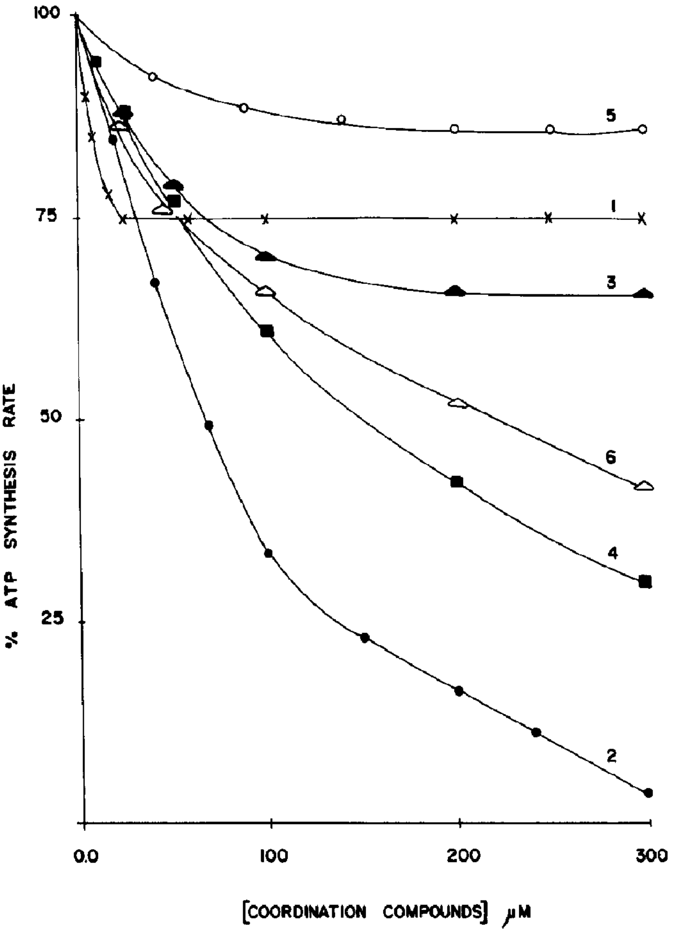

Fig. 3. Inhibition of non-cyclic ATP-synthesis in spinach thylakoid by quinic acid and its coordination compounds. (×) **1**, (●) **2**, (▲) **3**, (■) **4**, (○) **5**, (△) **6**. Methylviologen was used as electron acceptor and was determined as described in text. The reaction medium (3 mL) was 100 mM KCl, 5 mM $MgCl_2$, 1 mM Na^+-tricine (pH 8.0), 1 mM ADP, 3 mM potassium phosphate (pH 8.0) and 50 μM methylviologen. An amount of thylakoids equivalent to 50 μg of chlorophyll was used per chamber tested.

buffering medium and water). It was observed that since the UV–Vis spectra of all the complexes remained unchanged for 60 min at 25°C, both in water and in the buffering medium, the complexes did not undergo any hydrolysis or ligand substitution reaction in water or the buffering medium. On the other hand, the spectra in solution were compared with those in solid state and it was observed that the shape of the spectra and their maximum absorbance wavelengths are the same for both conditions, see Fig. 2 as an example. Their spectra were also compared with that of **1**. Quinic acid has an absorption band in the UV region with a peak at 224 nm and a shoulder at 210 nm, due to the $\pi^* \leftarrow \pi$ assigned to the C=O of the carboxylic group (Fig. 2), all the coordination complexes also show the expected *d–d* bands. Spectroscopic data for the coordination compounds are shown in Table 1. It was also observed that the buffer did not interact with the metal chlorides. We could therefore use these complexes in the buffering medium for essays in photosynthesis knowing that the structure of the coordination compound remains unchanged in solution at least for the time interval required for doing the experiment.

In order to know if metal chlorides, quinic acid or its coordination compounds affect photosynthesis, the following biochemical activities were measured for each of them on freshly lysed

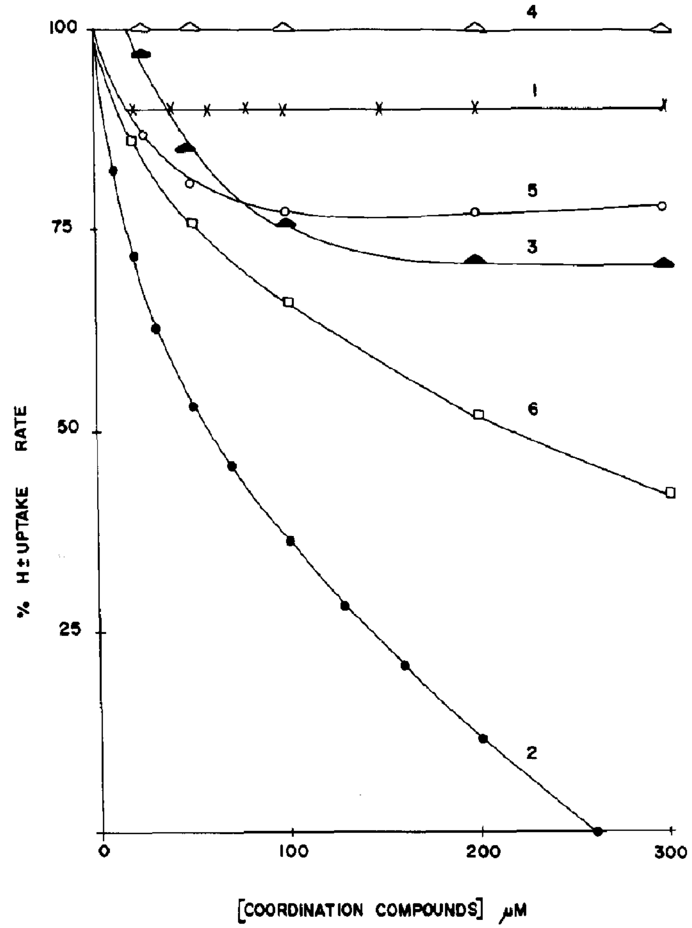

Fig. 4. Proton uptake dissipation by quinic acid and its coordination compounds. (×) 1, (●) 2, (▲) 3, (△) 4, (○) 5, (□) 6. The pH changes of thylakoid suspensions were measured with a pH-meter equipped with a combination electrode and a recorder. The reaction medium (3 mL) was 100 m*M* KCl, 5 m*M* $MgCl_2$, 50 μg of chlorophyll. Other conditions as described in text.

Table 1.
Diffuse reflectance (350–800 nm) and solution spectra (190–820 nm) for some coordination compounds of quinic acid

Compound	Electronic spectra	
	Diffuse reflectance λ_{max} (nm)	Aqueous solution λ_{max} (nm)
2 $[Co(quin)Cl(H_2O)]\cdot(H_2O)$	528 [a]	510 [a]
3 $[Co(quin)_2]$	532 [2]	515 [a]
4 $[Ni(quin)Cl(H_2O)]\cdot(H_2O)_2$	388, 678 [b]	392, 680 [b]

[a] Transition assigned as: $\nu_3 = {}^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$.

[b] Transitions assigned as: $\nu_3 = {}^3T_{1g}(P) \leftarrow {}^3A_{2g}$ and $\nu_2 = {}^3T_{1g}(F) \leftarrow {}^3A_{2g}$.

spinach chloroplasts: ATP-synthesis, H^+-uptake and electron transport rate (basal, phosphorylating and uncoupled) [5,11]. In each case a blank experiment was performed with the chloroplasts alone in the reaction medium. These experiments gave clue on the mechanism of action, i.e., if they behave as uncouplers, as Hill's reaction inhibitors, or as energy transfer inhibitors.

Photosynthesis can be affected by chemical substances, either inhibiting or activating it. In this context, quinic acid may act as decoupler based on its chemical structure, our results corroborated this statement (Figs. 3–6).

The quinic acid coordination compounds showed the following behaviour. Photosynthetic phosphorylation associated with the methylviologen (MV) reduction (Hill's reaction) in spinach thylakoid was 91% inhibited by compound **2** in concentrations up to 260 μM, while the other coordination compounds tested in this study only partially inhibited ATP-synthesis in concentrations up to 300 μM. The inhibition order was as follows: **2** (91%), **4** (70%), **6** (58%), **3** (35%) and **5** (14%). These results are particularly important due to the fact that quinic acid inhibits this activity 25% at 40 μM and the inhibition percentage remains constant up to 300 μM, whereas the coordination compounds are more potent inhibitors to photophosphorylation, i.e., coordination with metal ions potentiates its activity (Fig. 3).

The light dependent H^+-uptake (Fig. 4) was dissipated by the compounds in the following order: **2** (100%), **6** (58%), **3** (31%), **5** (22%), **1** (10%), and **4** (0%). The shape of the curves of activity percentage versus concentration paralleled those of ATP-synthesis, but the inhibition order for compounds **3** to **6** (Figs. 3 and 4) was different. These results indicate that compounds **3–6** affect photosynthesis through more than one mechanism, i.e., they can behave as decouplers and at the same time as Hill's reaction inhibitors or energy transfer inhibitors, for example. Compound **2** showed 100% H^+-uptake inhibition at 260 μM, compared to ATP-synthesis inhibition (91%, even at higher concentrations) the difference between the values for these two activities is not in agreement with Mitchell's transduction theory [12], according to which the same deactivation is expected for these two activities. The results presented here are in accord with the findings of other authors [13] that suggest that H^+-ATPase consumes the free energy that is stored in the proton pool embedded inside the thylakoid membrane, therefore ATP-synthesis can still take place.

The light dependent inhibiting effect of the ATP-synthesis and H^+-uptake on illuminated thylakoids can take place by three different mechanisms: (a) blockage of the electron transport, (b) inhibition of the phosphorylation reaction (energy transfer inhibitor), and (c) decoupling of ATP-synthesis from the electron transport.

To obtain further information about the mechanism of action of quinic acid and its coordination compounds, we studied the effect of all of them on electron transport. Electron transport rate is studied under three different conditions: basal, phosphorylating and decoupled. On addition of ADP and phosphate to the buffering medium, ATP-synthesis takes place using up the proton electrochemical gradient; when NH_4Cl is added to this medium phosphorylation is decoupled from Hill's reaction. With the information from these experiments we may clarify the type of action.

Phosphorylating electron transport rate from water to methylviologen increases with the concentration of quinic acid and its metal complexes, as shown in Fig. 5. The activity percentage of the coordination compounds are compared at a con-

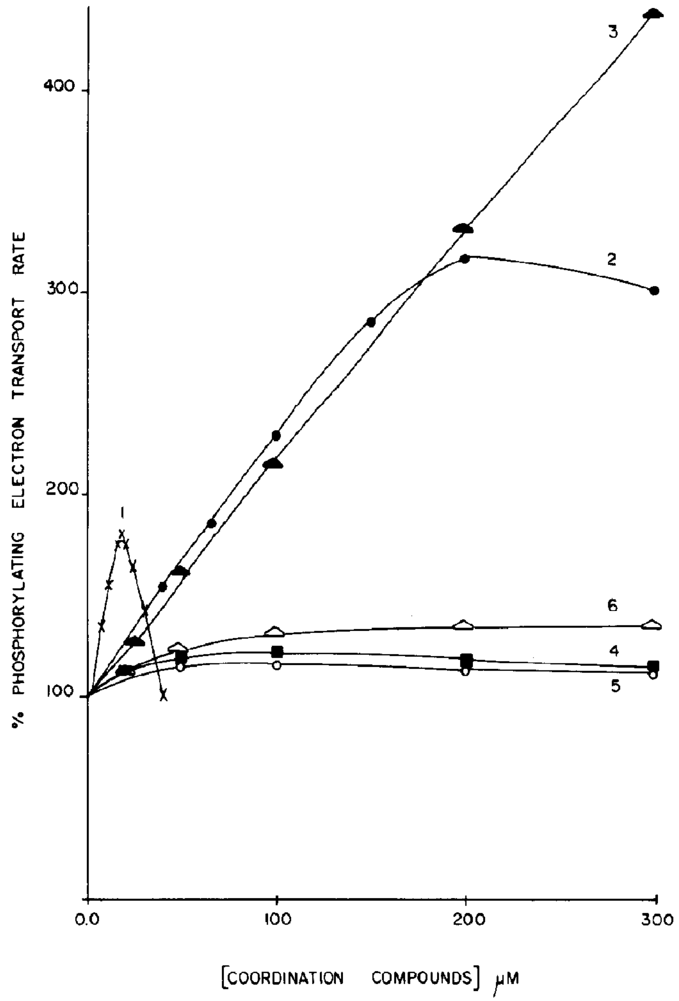

Fig. 5. Effect of quinic acid and its coordination compounds. (×) **1**, (●) **2**, (▲) **3**, (■) **4**, (○) **5** and (△) **6** on phosphorylating electron transport. Electron transport from water to methylviologen was measured as oxygen uptake with a Teflon-covered Clark electrode. The reaction medium (3 mL) was 100 mM sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 30 mM Na^+-tricine (pH 8.0), 50 μM methylviologen, and thylakoids (50 μg of chlorophyll), besides 1 mM ADP and 3 mM potassium phosphate were added.

centration of 300 μM, with exception of quinic acid (18 μM). The following order of activity was observed: **3** (340%), **2** (200%), **1** (80%, 18 μM), **6** (36%), **4** (14%), **5** (12%).

Basal electron transport rate was measured and the results obtained were: **3** (340%), **2** (87.5%), **1** (75%), **5** (21%), **4** (20%) and compound **6** is inactive (Fig. 6). Notice that the curve of quinic acid in Figs. 5 and 6 has a Gaussian shape, as expected for classical and non-classical decouplers [5,13]. At concentrations higher than 18 μM quinic acid inhibits basal and phosphorylating electron transports. This behaviour is due to the fact that decouplers inhibit water splitting enzymes, by blocking the electron flow from water to the PSII reaction centre, therefore the photolysis of water does not take place. Decouplers reduce ΔpH established in the light and lead to an elevation of the internal pH to a level sufficient to inactivate the O_2-evolving apparatus [14, and references therein]. The rest of the tested compounds should show the same behaviour at higher concentrations.

To complete this study uncoupled electron transport rate was determined in the presence of 5 mM NH_4Cl as a function of the concentration of the compounds. This activity was not affected by any of the coordination compounds (data not shown).

For quinic acid and all the coordination compounds, H^+-uptake and ATP-synthesis are inhibited, basal and phosphorylating electron transport rate are enhanced while decoupled electron transport rate is unaffected. These results clearly indicate that these compounds act as decouplers of photophosphorylation [5,13]. The experimental results showed that the order of inhibition or enhancement varies from one activity to another for the different tested compounds. It can be observed in Fig. 3 that extent of inhibition of

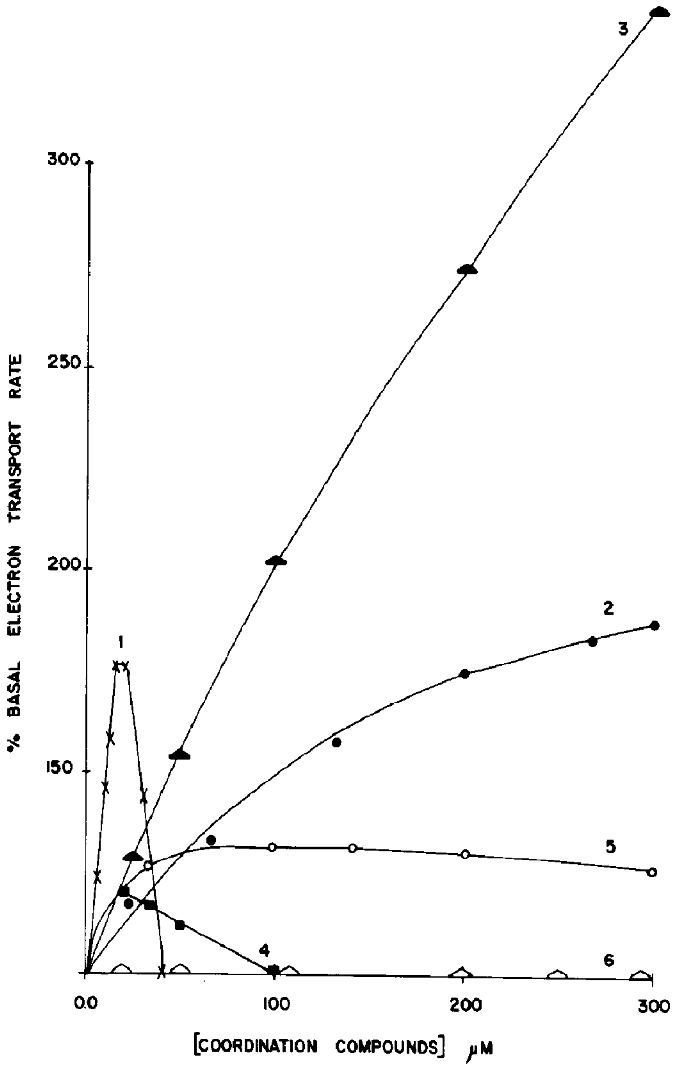

Fig. 6. Effect of quinic acid and its coordination compounds. (×) 1, (●) 2, (▲) 3, (■) 4, (○) 5, and (△) 6 on basal electron transport. Electron transport from water to methylviologen was measured as oxygen uptake with a Teflon-covered Clark electrode. The reaction medium (3 mL) was 100 m*M* sorbitol, 5 m*M* $MgCl_2$, 10 m*M* KCl, 30 m*M* Na^+-tricine (pH 8.0), 50 µ*M* methylviologen, and thylakoids (50 µg of chlorophyll).

ATP-synthesis is different from the extent of enhancement for the rate of phosphorylating electron transport (Fig. 5), for all the compounds. This can be explained by a non-classical decoupling behaviour, with the exception of **2** [5,13].

Coordination compound **2** is the most active decoupler of all the complexes tested.

It is also noteworthy that the slopes of the curves in Figs. 3 to 6 change with concentration showing biphasic behaviour. This result may indicate that all the coordination compounds may act with more than one mechanism of action when affecting these activities.

From these studies it is concluded that the nature of the metal ion is important in the activity of the complex, as can be observed that the most potent compounds contain cobalt(II). We may think that $[Co(quin)Cl(H_2O)] \cdot H_2O$ can suffer substitution reactions inside the chloroplast due to the presence of the labile water molecule ($k_{ex}(H_2O)$ for $[Co(H_2O)_6]^{2+} = 3.2 \times 10^6$ s^{-1} [15]) and chloride as ligands, or else the complex may release a proton producing the hydroxo species. Mitchell's hypothesis [12], related with the mechanism of action of the uncouplers, states that these species are proton carriers, complex **2** may act as proton carrier supporting this hypothesis.

Complex **2** shows the greatest protonophoric activity, while compound **4** does not present any and the rest are intermediate, as can be appreciated in Fig. 4. The H^+-uptake from outside the thylakoidal membrane into its interior is dissipated to the greatest extent by compound **2**.

In this research, copper coordination compounds of quinic acid were also studied and they did not present uncoupling properties. They behave as Hill's reaction inhibitors, the results of the target site characterization have been submitted for publication.

The effect of metal chlorides (within the concentration range 0–300 μM) on photosynthetic activities was also studied under the same working conditions (on freshly lysed chloroplasts), for comparison with the coordination compounds and quinic acid. In this work, it was found that zinc, cadmium and nickel chlorides have no effect on photosynthesis, on the other hand, cobalt chloride is a very mild decoupler (requires a concentration greater than 250 μM to show any activity). Data not shown.

These results are different to those found on previously published papers by other authors [16,17], who reported that metal salts act as Hill's reaction inhibitors. The difference in behaviour might be due to the fact that for these experiments washed thylakoids were used, and the chloroplast components present in the stroma (such as nucleic acids, enzymes, ribosomes, etc.) are removed; while the experiments reported by us were carried out on freshly lysed intact chloroplasts where the metal salts may interact with the different components of the stroma and lead to a different pattern of behaviour. This means that the tested salts can be partitioned among the stroma components and consequently only a small amount of the tested salt reaches the target thylakoid membranes.

4. Conclusions

This is the first report where quinic acid, a metabolite of shikimic acid pathway presents an uncoupling property in cloroplasts.

Quinic acid coordination compounds **1–6** behave as decouplers [6,11,13,14], complex **2** being the most potent. As far as we know, this is the first time where metal complexes are studied and behave as non-classical decouplers.

With these results we propose that quinic acid may present uncoupling properties *in vivo*. This activity is important in the regulation of energy metabolism in plants.

Metal chlorides do not behave either as uncouplers or Hill's reaction inhibitors on freshly lysed chloroplasts, except for cobalt chloride that acts as a mild uncoupler.

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