# ON THE MECHANISM OF ACTION OF SILICOMOLYBDIC ACID IN CHLOROPLASTS

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## 1. Introduction

Giaquinta et al. [1] described recently an electron acceptor system in isolated chloroplasts, comprised of silicomolybdic acid plus ferricyanide whose reduction was insensitive to 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU). According to these authors silicomolybdic acid is reduced by Q and reduces chemically ferricyanide.

Girault and Galmiche studied previously [2,3] the interaction of another heteropolyion, silicotungstic acid, with the chloroplast membrane. These authors concluded [3] that in the presence of silicotungstic acid the chloroplast membrane is changed and consequently ferricyanide is photoreduced in a DCMU insensitive reaction.

In this study we have shown: (a) that pretreating the chloroplasts with silicomolybdic acid and removing the bulk of it, causes a change in the properties of the electron transport chain; such chloroplasts can photoreduce ferricyanide (and other electron acceptors) in a DCMU insensitive reaction; (b) that the changes in the chloroplast membrane by pretreatment with silicomolybdic acid require specific conditions and (c) the chloroplasts in which Q has been exposed by silicomolybdic acid can photoreduce this compound.

## 2. Materials and methods

Chloroplasts were isolated as previously described [4], and chlorophyll was assayed after Arnon [5]. Standard reaction mixture contained 33 mM buffer and 33 mM NaCl. Tricine-maleate was used as a buffer in the pH range 5.5–8.5 and bicarbonate at pH values above 8.5. Silicomolybdic acid was kindly provided by Dr R. A. Dilley. Electron transport was measured as oxygen evolution, or as oxygen uptake, using a Yellow Springs Instruments Clark type oxygen electrode.

## 3. Results and discussion

The reduction of silicomolybdic acid as a function of pH by isolated chloroplasts is shown in fig.1. It can be seen that silicomolybdic acid is reduced at pH 7.0 at a rate of 400 μeq (electrons) per mg chlorophyll per hr. Photosystem 1 is obviously not involved in this reaction since the reduction is insensitive to dibromothymoquinone (DBMIB) which was shown to inhibit electron transport prior to Photosystem 1 [6]. The photoreduction of silicomolybdic acid is inhibited by DCMU at pH values of 8 and above, but at low pH values it is strikingly resistant to this inhibitor.

In order to find out whether silicomolybdic acid serves as a unique electron acceptor (in being insensitive to DCMU) or whether it changes the properties of the electron transport chain, chloroplasts were incubated with silicomolybdic acid, the acceptor was washed out and the effect of DCMU on the photoreduction of ferricyanide was determined. The results presented in table 1 show that in chloroplasts which were preincubated with silicomolybdic acid, under certain conditions, the photoreduction of ferricyanide becomes DCMU-resistant. Preincubation with silicomolybdic acid in the dark, either at pH 6.0 or at pH 8.0, causes a change, after which, the photoreduction of ferricyanide becomes DCMU insensitive. However, if the preincubation with silicomolybdic acid is carried

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Table 1
Effect of various conditions during silicomolybdic acid pretreatment on DCMU insensitive photoreduction of ferricyanide

| Conditions of preincubation pH | Silicomolybdic acid (mM) | | Oxygen evolution ($\mu$eq/mg chlorophyll × hr) | +DCMU |
|---|---|---|---|---|
| 6.0 | 0 | Dark | 100 | <20 |
| 8.0 | 0 | Dark | 124 | <20 |
| 6.0 | 0.1 | Dark | 144 | 132 |
| 6.0 | 0.1 | Light | 117 | 106 |
| 8.0 | 0.1 | Dark | 188 | 165 |
| 8.0 | 0.1 | Light | 102 | 38 |

The preincubation medium contained 33 mM Tricine – maleate at the indicated pH, 33 mM NaCl, 0.1 mM silicomolybdic acid where indicated and chloroplasts containing 30 $\mu$g chlorophyll/ml. Saturated intensity of white light was irradiated for 30 sec where indicated, and the silicomolybdic acid was added just before the light was turned on. The chloroplasts were centrifuged down and resuspended in 0.4 M sucrose, 0.01 M NaCl and 0.05 Tricine–NaOH pH 8.0. The reaction mixture for ferricyanide photoreduction contained 33 mM Tricine–maleate pH 7.1, 33 mM NaCl 0.6 mM ferricyanide and 2 $\mu$M DCMU where indicated. Chloroplasts contained 28–33 $\mu$g chlorophyll/ml for the different preparations.

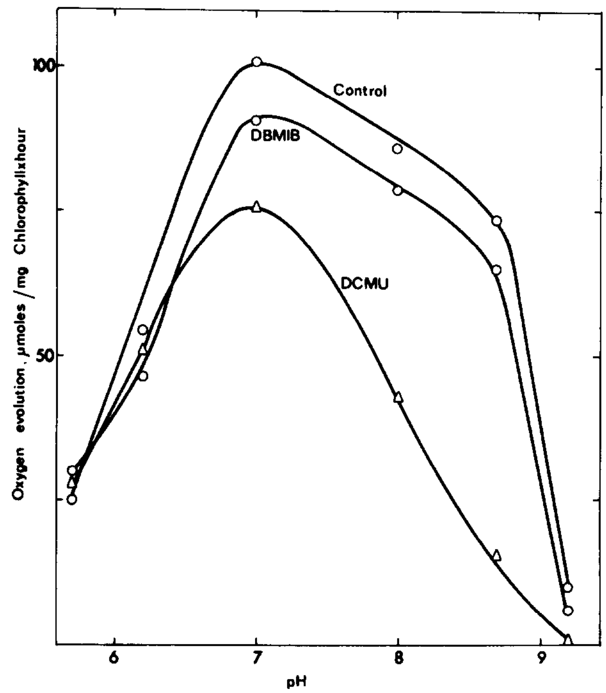


Fig.1. Effect of DCMU and DBMIB on silicomolybdic acid photoreduction as a function of pH. Standard reaction mixture as described under Methods. Chloroplasts contained 21 $\mu$g chlorophyll/ml. The concentration of silicomolybdic acid, DCMU and DBMIB were 100, 3.3 and 0.7 $\mu$M, respectively.

out in light, the pH of the preincubation medium becomes a decisive factor. At pH 6.0, pretreatment in light has the same effect as pretreatment in dark, whereas at pH 8.0 pretreatment in light has no effect, and ferricyanide photoreduction remains DCMU sensitive resembling control chloroplasts. These results agree well with the results of fig.1, in which silicomolybdic acid photoreduction by itself, when measured directly, was found to be insensitive to DCMU at pH 6.0 but inhibited by this inhibitor at pH 8.0.

The possibility that after pretreatment with silicomolybdic acid the residual amount of silicomolybdic acid acts 'catalytically' in the presence of ferricyanide i.e. it is reduced by the chloroplasts and reduces chemically ferricyanide can be ruled out. In such a system the rate of ferricyanide reduction should be determined by the rate of photoreduction of bound silicomolybdic acid, The results presented in table 2 show that in the presence of low concentration of silicomolybdic acid (and in the presence of DCMU) addition of ferricyanide does not stimulate the rate of electron flow obtained with silicomolybdic acid only. However, the rate of electron flow obtained after silicomolybdic acid pretreatment (with 0.1 mM silicomolybdic acid) is markedly stimulated upon addition of ferricyanide. Thus, it can be concluded that as a result of the silicomolybdic acid pretreatment, Q is exposed and consequently both the 'residual' amount of silicomolybdic acid and ferricyanide can be photoreduced by Photosystem 2 in a DCMU insensitive reaction.

From these data it may be concluded that silicomolybdic acid (in the dark) causes a change in the membrane by exposing Q to exogenous electron acceptors. Since this change does not take place in the light (at pH 8.0), the interaction of silicomolybdic acid with the chloroplast membrane may provide a useful tool for studying conformational changes around Photosystem 2.

As a result of the interaction of silicomolybdic acid with the electron transport chain, ferricyanide photoreduction is no longer DCMU-sensitive. This

Table 2
The effect of ferricyanide on the rate of electron transport at various concentrations of silicomolybdic acid and after silicomolybdic acid pretreatment

| Silicomolybdic acid mM | Control chloroplasts | + ferricyanide | Silicomolybdic washed | + ferricyanide |
|---|---|---|---|---|
| | ($\mu$eq/mg chlorophyll × hr) | | | |
| 0 | <20 | <20 | 60 | 204 |
| 0.01 | 33 | 27 | 102 | 219 |
| 0.02 | 45 | 54 | 125 | 220 |
| 0.04 | 115 | 160 | 153 | 230 |
| 0.10 | 262 | 300 | 250 | 250 |

The pretreatment of chloroplasts with silicomolybdic acid was carried out at pH 8.0 in the dark. Other conditions as in table 1. Reaction mixture contained 33 mM Tricine–maleate pH 7.5, 33 mM NaCl, 0.6 mM ferricyanide where indicated, 2 $\mu$M DCMU and chloroplasts containing 22 $\mu$g and 30 $\mu$g chlorophyll/ml for the control and silicomolybdic acid pretreated, respectively.

could be due to the 'destruction' of the DCMU inhibitor site. However, the data presented in table 3 eliminate this possibility. In these data, it is shown that in chloroplasts pretreated with silicomolybdic acid in the dark, 2 $\mu$M DCMU inhibits very slightly the photoreduction of ferricyanide, but it inhibits completely the photoreduction of methyl viologen. These results resemble the data presented by Girault and Galmiche [3] who showed that addition of silicotungstic acid to chloroplasts has no effect on the binding of DCMU. It is of interest to note that exposure of Q does not interfere with electron flow to Photosystem 1.

Chloroplasts pretreated with silicomolybdic acid (in which Q is exposed) are able to photoreduce dichlorophenol-indophenol in a DCMU-insensitive reaction.

In conclusion silicomolybdic acid exposes Q to exogenous electron acceptors, whose reduction is insensitive to DCMU. Such chloroplasts can reduce silicomolybdic acid, ferricyanide and probably other electron acceptors as well.

Table 3
The sensitivity of ferricyanide and methyl viologen photoreduction towards DCMU in silicomolybdic acid pretreated chloroplasts

| Conditions of preincubation: Silicomolybdic acid (mM) | | Ferricyanide reduction | +DCMU | Methyl viologen reduction | +DCMU |
|---|---|---|---|---|---|
| | | ($\mu$eq/mg chlorophyll × hr) | | | |
| 0 | Dark | 274 | <20 | 174 | <20 |
| 0.1 | Dark | 260 | 218 | 202 | <20 |
| 0.1 | Light | 246 | 30 | 202 | <20 |

Preincubation of the chloroplasts as described in table 1, the pH was 8.0. Methyl viologen photoreduction was measured as oxygen uptake and 0.2 mM methyl viologen and 1 mM Na-azide replaced ferricyanide. The pH of the reaction mixture was 7.5, other conditions as in table 1.

## References

[1] Giaquinta, R. T., Dilley, R. A., Crane, F. L. and Barr, R. (1974) Biochem. Biophys. Res. Commun. 59, 985–991.
[2] Girault, G. and Galmiche, J. M. (1972) FEBS Lett. 19, 315–318.
[3] Girault, G. and Galmiche, J. M. (1974) Biochim. Biophys. Acta 333, 314–319.
[4] Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151.
[5] Arnon, D. I. (1949) Plant Physiol. 24, 1–15.
[6] Trebst, A., Harth, E. and Draber, W. (1970) Z. Naturforsch. 25b, 1157–1159.