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MONOVALENT ANION INTERACTION ON THE ELECTRON FLOW OF PHOTOSYSTEM II IN SOYABEAN CELLS

By

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تأثير تفاعل الأنيونات الأحادية العكسي على الإنتقال الإلكتروني في النظام الضوئي الثاني باستخدام خلايا نبات فول الصويا

فاطمـــة على الشنتناوي

تهدف هذه الدراسة إلى إثبات وجود ظاهرة التفاعل الأنيوني العكسي ممثلا في أنيونات الفورمات والنيترات والبيكربونات في خلايا الأنسجة المستنبته من نبات فول الصويا . هذه الظاهرة معروفة بتأثيرها على الانتقال الالكتروني في تفاعلات النظام الضوئي الثاني لعملية البناء الضوئي . وقد أعتمدت الوسائل المستخدمة لأثبات تلك الظاهرة على قياس معدل الانتقال الالكتروني خلال النظام الضوئي الثاني بالاستعانة بطرق التفلور (باستخدام طريقة الوميض الثنائي) وكذلك قياس معدل الأكسجين المتصاعد بواسطة الكترود كلارك .

عند قياس منحنى التفلور في خلايا نبات فول الصويا والمعالجة بواسطة محلول يحتوى على أنيون الفورمات أو النيترات لوحظ إنخفاض في معدل إنحسار التفلور للكلوروفيل أ مما يشير إلى إبطاء معدل أكسدة المستقبل الالكتروني الأولى للنظام الضوئي الثاني والمعروف باسم بلاكستوكينون أ عند إضافة أنيون البيكربونات لتلك الخلايا المعالجة يزداد معدل انحسار التفلور للكلوروفيل أ مما يدل على زيادة معدل أكسدة المستقبل الأولى المختزل وبالتالي سرعة إنتقال الالكترونات إلى المستقبل الالكتروني الثانوي للنظام الضوئي الثاني والمعروف باسم بلاستوكينون ب من المستقبل الأولى . في حالة إضافة أنيون البيكربونات لخلايا لم يسبق معالجتها بأي من الفورمات أو النيترات يزداد معدل أكسدة المستقبل الالكتروني الأولى بنسبة تصل إلى نصف المعدل الطبيعي فقط .

عند قياس معدل الاكسجين المتصاعد من خلايا نبات فول الصويا المعالجة إما بأنيون الفورمات أو النيترات لوحظ انخفاضا ملموسا الذي سرعان ما يصل إلى أعلى معدلات تصاعده والتي تساوي نسبة تصاعده في الخلايا الغير معالجة أنيونيا عند إضافة أنيون البيكربونات . عند قياس نشاط تفاعل (هيل) في خلايا لم يسبق معالجتها أنيونيا وعوملت فقط بأنيون البيكربونات لوحظ أن نسبة الاكسجين المتصاعد في ذلك التفاعل تعادل ٥٠٪ من النسبة المعتاده الانطلاق من الخلايا الغير معالجة أنيونيا .

مما سبق يتضح أن كل من أنيوني الفورمات والنيترات لهما تأثير مثبط على الانتقال الالكتروني خلال النظام الضوئي الثاني في عملية البناء الضوئي . أما أنيون البيكربونات ذات التأثير العكسي فيعتبر منشط للانتقال الالكتروني في تفاعلات النظام الضوئي الثاني لذلك فهو أنيون متميز وفريد التأثير بين جميع الأنيونات الاحادية . بناءا على ما أظهرته النتائج السابقة فإن البحث يثبت بطريقة واضحة وجود ظاهرة التفاعل الأنيوني العكسي في خلايا الانسجة المستنبته من نبات فول الصويا كما أثبت وجودها سابقا في البلاستيدات المستخلصة من النباتات الراقية .

Key Words: Chlorophyll *a* fluorescence, Oxygen evolution, Reversible anion interaction, Soybean cells.

ABSTRACT

Steady state of electron transport measurements from water to DMQ (2, 5 - dimethyl - P- benzoquinone) show a maximum bicarbonate effect (about four fold stimulation) following the addition of 5 mM HCO^-_3 to the previously 25 mM (formate or nitrate) treated cells at pH 6.7. However, addition of 5 mM bicarbonate to the non-anion treated cells (the cells were exposed for 150 min to nitrogen gas only) produced about 70% restoration compared to the anion-treated cells. Increasing the incubation time up to 3 hours decreased the restoration to 43%. Thus, exposing the cells to N_2 gas in the absence of the inhibitory anions caused an irreversible damage of photosystem II reflected by the linear electron transport from H_2O to DMQ. Studying the kinetics of Q_A^- reoxidation (Q_A is the primary quinone electron acceptor of photosystem II) of the anion treated cells shows that nitrite and formate can substitute each other in replacing bicarbonate between Q_A^- and Q_B (Q_B is the secondary quinone electron acceptor of photosystem II) and to the plasto-quinone pool. Analysis of chlorophyll *a* fluorescence decays of non-anion treated and HCO^-_3 restored cells demonstrates a remarkable irreversible inhibition of the electron flow between Q_A^- and Q_B at the electron acceptor side of photo-system II. Thus, the anion interaction on the electron transport is suggested to be located at the level of the two electron gate of photosystem II in the intact soybean cells.

INTRODUCTION

The phenomenon of the reversible anion effect on the electron flow at the acceptor side of photosystem II has been studied extensively by many workers in higher plant thylakoids. Originally, Warburg and Kripphal [1] showed the stimulatory role of bicarbonate on the Hill reaction. Good [2] was the first to test the reversible anion interaction in chloroplasts and he found that monovalent anions particularly formate and acetate, in contrast to bicarbonate, inhibit the electron flow of photosystem II. Stemler and Murphy [3] have demonstrated that nitrite is a more effective competitor of bicarbonate binding than formate. Eaton-Rye *et al.* [4] have reported that bicarbonate depletion can inhibit the steady state of electron transport supported by methyl viologen when nitrite was used instead of formate in both the depletion and reaction media. Blubaugh and Govindjee [5] took the advantage of the pH dependent Hill reaction on the ratio of HCO_3^- to CO_2 at equilibrium and showed that HCO_3, not CO_2, is the active species that binds to the effector site in the thylakoid membrane. Jusinic *et al.* [6] indicated that bound bicarbonate is believed to be essential for the normal rate of the electron flow from Q_A^- to Q_B and to the plastoquinone pool. Eaton-Rye and Govindjee [7, 8] have shown that reoxidation of Q_A^- decay was dramatically slowed down in formate treated thylakoid membranes. However, addition of bicarbonate to the formate treated samples restored the inhibition in the electron flow and produced a faster decay which is similar to the control. Graan and Ort [9] explained the slowing of the Q_A^- decay caused by bicarbonate depletion as a result of some inactive photosystem II centers in the membranes. The slowing of Q_A^- reoxidation in the anion treated membranes has been measured by the absorbance change at 320 nm by Farineau and Mathis [10] and at 515 nm in intact chloroplasts by Van Rensen and Snel [11]. El-Shintinawy and Govindjee [12] observed two sites of reversible anion interaction at the acceptor side of photosystem II; one between Z (A is the electron donor to photosystem II reaction center) and Q_A and the other from Q_A^- to Q_B using intact leaves. The above mentioned reversible anion effects have been observed also in intact algal cells by El-Shintinawy *et al.* [13]. This work was carried out to demonstrate the reversible anion interaction (bicarbonate, formate and nitrite) in the intact system soybean cells as shown earlier in thylakoid membranes. The ability of some monovalent anions (formate and nitrite) to replace each other in cells incubated for different times in anion containing media at a wide pH range has been studied. The role of HCO_3^- in reversing the inhibitory effect of HCO_2^- and NO_2^- was also shown. The inhibition of the electron flow of photo-system II resulting from the bicarbonate depletion procedure in the absence of the inhibitory anions (HCO_2^- and NO_2^-) and the restoration caused by bicarbonate addition were also investigated.

Materials and methods

Soybean cells, cell line sB-P (Corosoy), were cultured photoautotrophically as described by Horn *et al.* [14]. KN° medium was used as a culture medium as described by Rogers *et al.* [15]. The cells were grown for 14 days at 28°C in a 5% CO_2 atmosphere under continuous light of 300μ moles photons $m^{-2}s^{-1}$ and shaken at 130 rpm on a gyratory shaker. Chlorophyll concentration was determined by the spectrophotometric method of Arnon [16]. Anion treatment was carried out according to the method described by El-Shintinawy *et al.* [13]. Anion treated samples were prepared by incubating the cells in KN° medium containing different concentrations of formate or nitrite for different times and pHs at 20°C under a constant rate of N_2 gas. Bicarbonate restored samples were prepared by adding 5 or 20 mM HCO^-_3 to the anion or non-anion treated cells in the dark. Rates of oxygen evolution were determined polarographically at 25°C using a Clark electrode. Illumination was provided by a slide projector fitted with a Corning CS 3-68 yellow filter. Twenty μg Chl/ml of soybean cells were used for the oxygen evolution measurements. One mM 2,5-dimethyl -P- benzoquinone (DMQ) was used as artificial electron acceptor. One mM ferricyanide was used as an additional acceptor to keep the first acceptor oxidized. 0.5 μm 2,5-dibromo -6- isopropyl -P-benzoquinone (DBMIB) was added to the cell culture as an inhibitor between photosystem II and I. Kinetics of the decay of variable chlorophyll *a* fluorescence were measured at 685 nm (10 nm bandwidth) by a week measuring flash which was fired at variable times after each actinic flash. Both the actinic and measuring flash (Stroboslave 1593A, General Radio) were filtered with Corning blue (CS 4-96) filters [for more details see Eaton-Rye and Govindjee [7,8]. Ten μg Chl/ml of soybean cells were used for the fluorescence measurements.

RESULTS AND DISCUSSION

Anion effect on the steady state of electron flow from H_2O to DMQ

To evaluate the anion effect in the intact system soybean cells the factors affecting the anion interaction in the thylakoid membranes were investigated using cell cultures of soybean cells. The oxygen evolution rate in the anion treated membranes was found to be dependent on the anion incubation time as suggested by Eaton-Rye *et al.* [4]. The stimulation in Hill reaction activity by bicarbonate was found to be a pH dependent and HCO_3^- is the active species responsible for the stimulation of the electron flow of photosystem II as reported by Blubaugh and Govindjee [5] using thylakoid membranes. In intact systems, HCO_3^- is a source of carbon reduction beside its role in controlling the electron flow at the acceptor complex of photosystem II as shown by El-Shintinawy and Govindjee [17]. Therefore, the net stimulation on the electron transport free from its effect in CO_2 fixation was calculated in the presence of DBMIB which blocks the electron flow between the two photo-systems II and I as suggested earlier by Trebst *et al.* [18]. The steady state of electron transport from H_2O to DMQ was used to monitor the activity in anion treated and HCO_3^- restored cells. Fig. 1 shows the Hill reaction activity in soybean cells incubated for different times in culture media containing HCO_2^-, NO_2^- or in non-anion containing media at pH 5.8 in the presence of 1 mM DMQ and 1 mM ferricyanide as electron acceptors and 0.5 μm DBMIB as an inhibitor . The oxygen evolution rate reached its minimum value in about three hours when the culture medium contained 25 mM HCO_2^- or 25 mM NO_2^-. The rates were compared to those observed in control cells incubated at pH 6.5 in non-anion containing medium and equilibrated with air. A complete restoration of the Hill activity was observed upon bicarbonate (5 mM) addition to the anion treated cells (the top curves in Fig. 1). When HCO_2^- and NO_2^- have been removed from the reaction media and the cells were treated by bubbling with N_2, gas, the oxygen evolution was severely inhibited. However, non-anion treated cells failed to respond to added HCO_3^-; continued incubation beyond 150 min decreased the restoration from 75% to 43% compared to control. this observation is in agreement with the report of Fischer and Metzner [19] using argon gas to keep the depleted membranes of HCO_3^-. Fig. 2 shows the anion effect $+HCO_3^-$ /$-HCO_3^-$ on the oxygen evolution rate in culture media with different pHs. Addition of 5 mM HCO_3^- to the 25 mM HCO_2^- or 25 mM NO_2^- treated cells stimulated the electron transport rate depending on the pH of the reaction medium. A maximum HCO_3^- effect (4 fold in HCO_2^- treated and 3.6 in NO_2^- treated) was found to be at 6.7. These results support the notion that HCO_3^- may be the active species in the stimulation of Hill reaction which was discovered by Blugaugh and Govindjee (1986) using thylakoids. A relatively weak restoration in the electron flow in non-anion treated cells was recorded (1.5 fold). The results, shown here, are similar to those measured in HCO_3^- -restored to HCO_3^- -depleted chloroplasts by Van Rensen and Vermaas [20]. The above oxygen evolution measurements confirm the phenomenon of anion interaction on the electron transport of photosystem II in the previously anion and non-anion treated soybean cells as it has been observed in thylakoid membranes.

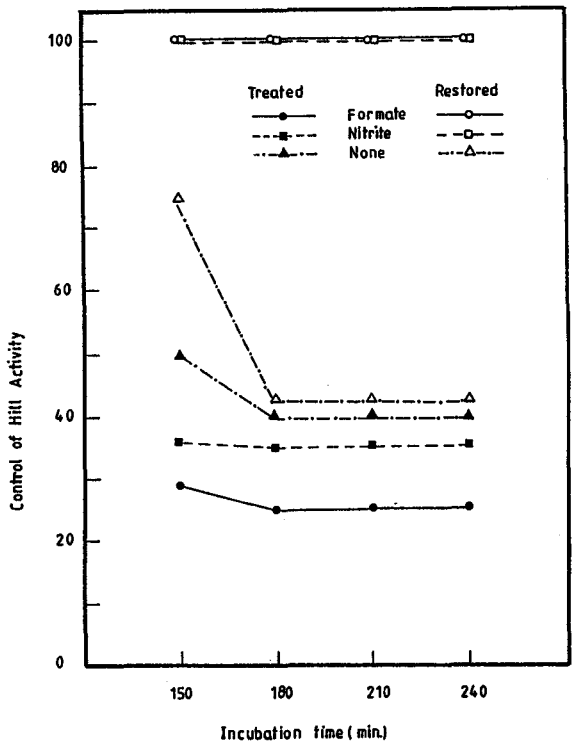


Figure 1: Hill reaction activity of soybean cells incubated (at pH 5.8) for different times in KN° media containing 25 mM HCO_2^- (●), 25 mM NO_2^- (■) or in non-anion containing media (). The restored rates (o, □ and ●) were prepared by adding 5 mM HCO_3^- to the treated cells. Cell suspension containing 20 μg Chl/ml were used. DMQ = 1mM, K_3 Fe (CN_6) = 1 mM and DBMIB = 0.5 μM.

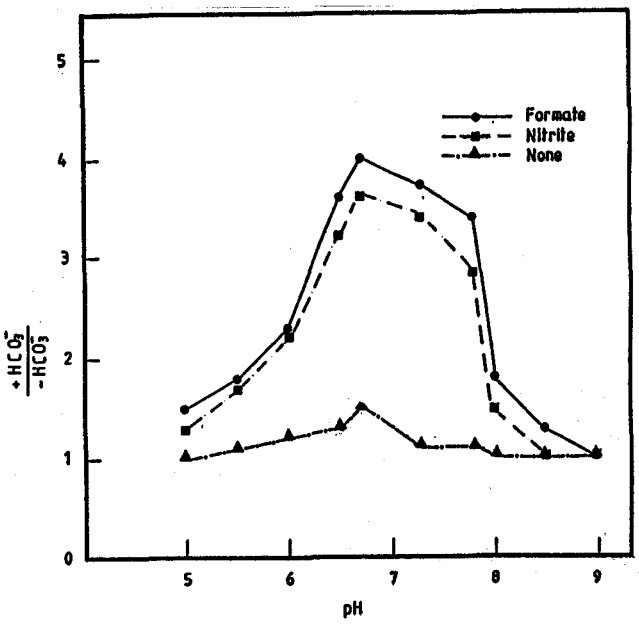
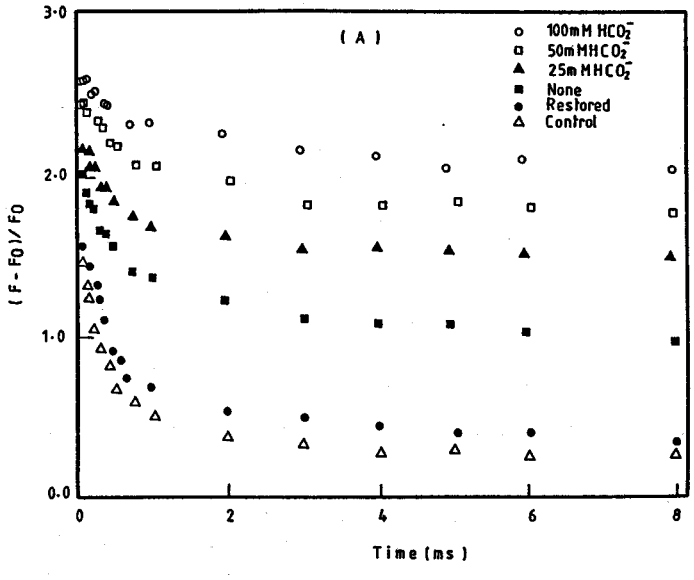


Figure 2: The ratio of the oxygen evolution in HCO_3^- restored to anion-treated cells as a function of pH. (●) represents data for 25 mM HCO_2^- -treated, (■) for 25 mM NO_2^- -treated and (Δ) for non-anion treated cells. The conditions were identical to Fig. 1 with the exception that the cells were incubated in KN° media for three hours at different pHs.

Anion effect on the kinetics of chlorophyll a fluorescence decays

Much information regarding the reversible anion effect in thylakoid membranes has been derived from studies of variable chlorophyll *a* fluorescence. Duysens and Sweers [21] explained the quenching of chlorophyll a fluorescence transient as a result of the oxidation of Q_A^-. They showed that the maximum fluorescence can be reached only when Q_As are fully reduced. Jursinic *et al.* [6] reported that chlorophyll a fluorescence decay in anion-treated (HCO_2^- or NO_2^-) membranes can be used to monitor the oxidation of Q_A^- indicating the inhibition in the electron flow at the acceptor side of photosystem II. HCO_3^- -restored membranes, in contrast to the anion-treated membranes, have faster chlorophyll *a* fluorescence decays reflecting the stimulation of the electron flow at the electron acceptor complex of photosystem II as mentioned by Govindjee and Eaton-Rye [22] Soybean cells were incubated in media containing different anion concentrations for three hours at pH 5.8 (for details see materials and methods). Fig. 3 shows that the chlorophyll *a* fluorescence decays, monitoring the oxidation of Q_A^- , after three actinic flashes at pH 6.5 in formate-treated (A) and nitrite-treated (B) cells. The rate of Q_A^- oxidation was greatly slowed down as the anion concentration increased reflecting the inhibition of the electron flow from Q_A^- to Q_B. Moreover, addition of 20 mM HCO_3^- to the 100 mM anion-treated (HCO_2^- or NO_2^-) cells restored the rate of the electron flow to normal. Incubating the cells in non-anion containing media produced a slower Q_A^- decay compared to control but it was faster than that of anion-treated cells.

Studying the Q_A^- decays with different anion treatment indicates that they are apparently biphasic: a fast component (the half-time in the us range) and an intermediate components (the half-time in the ms range). These two components were suggested by Robinson and Crofts [23] using spinach chloroplasts to be resulted from an altered equilibrium of Q_A^- with plastoquinone and plastoquinol at the Q_B binding site on the quinone acceptor complex of photosystem II. The half times and amplitudes of both fast (f) and intermediate (i) components of chlorophyll *a* fluorescence decays after three actinic flashes in soybean cells with different treatments at pH 6.5 are presented in Table 1.



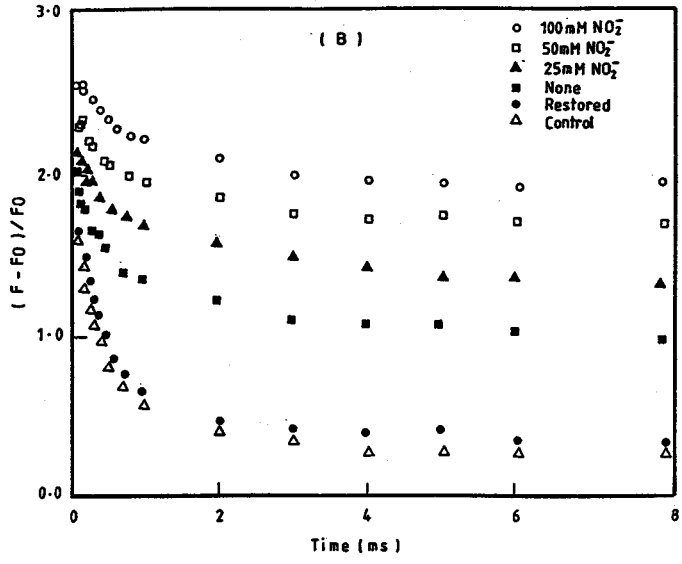


Figure 3: The effect of anion treatment on the decay of chlorophyll a fluorescence yield after the third actinic flash in formate-treated (A) or nitrite-treated (B) cells at pH 6.5 (o) represents data for 100 mM, (□) for 50 mM, (Δ) for 25 mM, (■) for non-anion treated, (●) for HCO_3^- -restored and (Δ) for control soybean cells.. Fo is the chlorophyll a fluorescence yield from the measuring flash with all Q_A^- oxidized and F is the yield at the indicated time the actinic flash.

Table 1

The effect of anion treatment on the ratios of the half-time (R_t) and the amplitude (R_A) of fast (f) or intermediate (i) components of chlorophyll *a* fluorescence decays (after three actinic flashes) of soybean cells. Both the half-times and the amplitudes were calculated from Q_A^- decay curves of soybean cells with different anion treatment at pH 6.5.

	Treated			
Ratio	Restored	HCO_2^-	NO_2^-	None
R_t (f)		2.1	2.6	1.1
R_t (i)		1.8	2.2	1.1
R_A (f)		0.2	0.3	0.1
R_A (i)		2.4	2.6	1.1

Both the half-times and the amplitudes are calculated from Q_A^- decays after three actinic flashes in soybean cells with different treatments at pH 6.5 are presented in Table 1. Both the half-times and the amplitudes are calculated from Q_A^- decay curves for 100 mM HCO_3^-, 100 mN NO_2^-, non-anion treated and 20 mM HCO_3^- restored soybean cells. The ratio of the half-times of both components (f and i) of decays in anion-treated to HCO_3^- restored cells is about twice the ratio in non-

anion treated cells. In the absence of the inhibitory anion, the ratio of the amplitudes (f and i) of non-anion treated to HCO_3^- -restored samples is about 2-3 times smaller than that in anion-treated cells. These results are in agreement with the report of Robinson *et al.* [24] suggesting that a major site of anion effect on the electron flow may be at the level of the two electron gate from Q_A^- to Q_B.

CONCLUDING REMARKS

The oxygen evolution measurements demonstrate a fully reversible anion interaction upon the addition of bicarbonate to the anion (formate or nitrite) treated soybean cells (Figs. 1 and 2). However, addition of bicarbonate to the non-anion treated cells produced a weak restoration in the Hill activity (Fig. 1) and a smaller ratio of the anion effect (Fig. 2) reflecting an irreversible inhibition of the electron flow of photosystem II. Analysis of chlorophyll *a* fluorescence decays confirms the notion that anion effect has to be located at the acceptor quinone complex of photosystem II. Nitrite, like formate, inhibits the electron flow from Q_A^- to Q_B as shown by the slowing of chlorophyll *a* fluorescence decays and thus Q_A^- oxidation (Fig. 3 A, B). Bicarbonate, unlike both nitrite and formate, stimulates the electron transfer between Q_A^- and Q_B as clearly demonstrated in Table 1 and Fig. 3. Therefore, bicarbonate which has a unique role among monovalent anions, is believed to be an activator of photosystem II in photosynthesis.

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