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IS ANTIMYCIN A A SPECIFIC INHIBITOR OF THE SLOW RISE OF THE ELECTROCHROMIC ABSORBANCE CHANGE IN INTACT CHLOROPLASTS?

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SUMMARY: The inhibitory effect of antimycin A on the slow rise of the flash-induced electrochromic absorbance change was reinvestigated in intact chloroplasts isolated from pea leaves. It is shown that in the absence of nigericin and ^{+}K at low repetition rates ($<0.5\ s^{-1}$) of the excitation flashes not only the slow ($\sim$10 ms) rise but also the initial ($<<$1 ms) rise generated by photosystem 1 is inhibited by antimycin A.

In algal cells (1,2) and in intact isolated chloroplasts (3-6) the initial ($<<$ 1 ms) rise of the electrochromic absorbance change is followed by a slower rise in the 10 ms time range. Although there is not doubt about the electrochromic nature of this kinetic component (7,8) it's origin is still debated (8-12). In the elucidation of this question inhibitors play an important role. Antimycin A is one of the inhibitors which have been shown to specifically inhibit the slow electrochromic rise in chloroplasts (13).

Previous experiments with antimycin A, however, were carried out in the presence of nigericin and ^{+}K-ions which were used to enhance the amplitude of the slow rise. However, since antimycin A is an inhibitory uncoupler (14) interference

Abbreviations used: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea

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of its action with that of the ionophoric uncoupler, nigericin, could not be excluded. Thus it was not redundant to investigate the effect of antimycin A on the electrochromic absorbance change in the absence of nigericin and ^{+}K. It is shown that antimycin A diminishes not only the slow rise but also the initial rise generated by photosystem 1.

MATERIALS AND METHODS

Intact chloroplasts from 2 week old pea seedlings were isolated according to reference (15). The absorbance changes at 515 nm at 15°C±1°C upon saturating flash excitation (>630 nm, 3 μs duration at half-peak emission) were determined in a set-up previously described (16).

The standard reaction medium contained 0.35 M sorbitol, 20 mM tricine and 5 mM $MgCO_3$, pH=7.7. The chlorophyll content of the samples was adjusted to about 40 μM.

RESULTS AND DISCUSSION

As seen in Fig. 1 antimycin A strongly affects the flash-induced electrochromic absorbance change in intact chloroplasts. With increasing concentrations of antimycin A the amplitude of both the initial and slow rise gradually decreased though to a different extent. (For clarity of the figure effect of antimycin A is shown only with two different concentrations.)

In chloroplasts pretreated with DCMU and dithionite the initial and slow rise were inhibited to about the same extent. In these chloroplasts, with silent photosystem 2 and with photosystem 1 driven by a proper redox poising (e.g. 8), 25 μM antimycin A caused about 40% decrease in the amplitude of both the initial and slow rise. In these samples the slow rise could not be completely (and independently of the initial rise) inhibited even in the presence of 50 μM antimycin A. For comparison the effect of 0.3 μM DBMIB is also shown in Fig. 2.

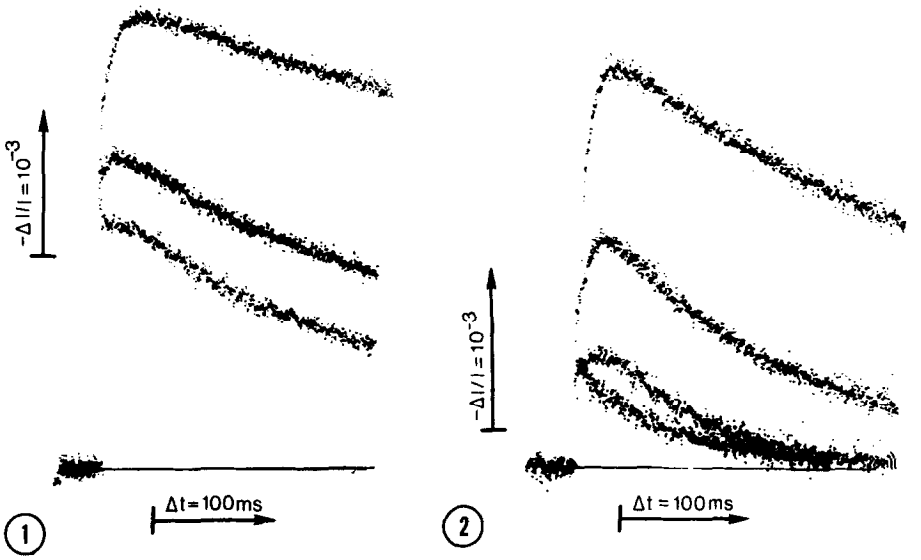

Figure 1. Effect of antimycin A on the flash-induced absorbance change at 515 nm in intact chloroplasts. Curves from the top to the bottom: without addition and in the presence of 15 μM and 40 μM antimycin A, respectively. Kinetic traces from 30 repetitions, Δt = 15 s dark period between the exciting flashes.

Figure 2. Effect of antimycin A on the flash-induced absorbance change at 515 nm in intact chloroplasts in the presence of 20 μM DCMU and 6 mM dithionite. Curves from the top to the bottom: without addition and in the presence of 25 and 50 μM antimycin A and 50 μM antimycin A plus 0.3 μM DBMIB, respectively. Kinetic traces from 30 repetitions Δt = 15 s dark period between the exciting flashes.

Based on these data it can be concluded that antimycin A is not a specific inhibitor of the slow electrochromic rise, but also inhibits the initial rise generated by photosystem 1 by about the same extent.

This conclusion is at variance with that of Shahak *et al.* (13). Based on results obtained in different experimental conditions (in the presence of nigericin and ^{+}K and at high repetition rates of the excitation flashes) they concluded that antimycin A inhibited specifically the slow rise.

The following factors could lead to the discrepancy between the results of Shahak *et al.* (13) and those of ours: 1/ The action of nigericin could interfere with that of antimycin A. 2/ Changes in the kinetic parameters upon addi-

tion of antimycin A could lead to an apparent decrease of the slow rise.

Antimycin A has been shown to act as uncoupler (14). At high concentrations (>10 μM) the ionophoric uncoupler nigericin, which at low concentrations enhances the slow rise can cause a breakdown of this kinetic component (Garab and Farineau, unpublished). Thus the first possibility cannot be excluded. On the other hand, the decay of the electrochromic absorbance change is strongly enhanced by antimycin A. This is indicative of its action as an uncoupler. Upon addition of 25 μM antimycin A at low ($f = 0.1\ s^{-1}$) and at high ($f = 1\ s^{-1}$) repetition rates of exciting flashes, the halftime of the decay decreased from about 700 ms to about 300 ms (cf. Fig.1) and from about 300 ms to about 150 ms, respectively. (At high repetition rates the experiments were carried out in the presence of 0.5-2 μM nigericin and 5-10 mM KCl.) Acceleration of the decay could already be observed at relatively low concentrations, 2-5 μM, of antimycin A (data not shown). Due to such acceleration of the decay the *apparent* amplitude of the slow rise decreased considerably. Most of the "difference kinetics", (-) antimycin A - (+) antimycin A, which were very similar to those obtained by Shahak *et al.* (13), could also be accounted for by the acceleration of the decay.

At present we have no definite explanation of the mechanism of action of antimycin A. However, it has been clearly demonstrated, that it does not specifically inhibit the slow electrochromic rise. On the other hand, it has been shown unambigously that antimycin A inhibits the cyclic electron-transport around photosystem 1 (4). Under our experimental conditions antimycin A also affected the kinetics of the cytochromes in the same way as shown by Slovacek and Hind (4).

Inhibition of the cyclic electron transport by antimycin A can be very likely correlated with the decrease of the initial rise of photosystem 1 and of the consecutive slow rise. Thus it follows that, in accordance with the model proposed by Crowther and Hind (8), cyclic regime of photosystem 1 plays an important role in the energization of the thylakoids.

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