Formate as an Inhibitor of Photosynthetic Electron Flow

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Z. Naturforsch. **39 c,** 386–388 (1984); received December 21, 1983

Photosynthesis, Hill Reaction, Formate, Bicarbonate, Inhibition

The effects of formate on the Hill reaction in isolated broken pea chloroplasts were investigated. Addition of formate to chloroplasts has two distinct effects: 1. basal electron flow can be stimulated 3-fold; 2. uncoupled electron flow is inhibited. The stimulating effect is due to uncoupling by formate and appears instantaneous. Maximal inhibition by formate is only observed after prolonged illumination. The inhibitory action of formate on electron flow can be relieved by bicarbonate*.

Introduction

Effects of formate on photosynthetic electron flow are known for some time. Isolated thylakoids that are incubated in media containing formate, are inhibited in their ability to produce oxygen in the presence of an artificial electron acceptor [1–3]. The inhibitory effect of formate is counteracted by bicarbonate*. As several other anions besides formate are also inhibitory and as bicarbonate* is the only compound that has been shown to relieve this inhibition [1], it was suggested that binding of bicarbonate* to "active sites" on the thylakoids is a requirement for the Hill reaction [4]. The stimulating effect of bicarbonate* on the Hill reaction is known as the "bicarbonate-effect" on electron flow. Although it has been proposed that bicarbonate* is involved in oxygen evolution [5, 6], the major site of the "bicarbonate-effect" appears to be located at the reducing side of photosystem II, between Q_A and PQ [7–9].

The reactivation of the Hill reaction by bicarbonate* in formate-containing media is dependent on the formate concentration [10]. Formate seems to be a competitive inhibitor of both the binding of bicarbonate* to thylakoids [8] and the reactivation of the Hill reaction by bicarbonate* in CO_2-depleted chloroplasts [11]. These experiments however refer to an interaction of bicarbonate* and formate at a regulatory site, perhaps on the Q_B-protein, in darkness. From a physiological point of view it is more interesting to study the interaction of formate and bicarbonate* on electron flow in the light, when electron flow is actually taking place. In this paper the effects of formate and bicarbonate* on the Hill reaction during prolonged illumination are reported.

Materials and Methods

Chloroplasts were isolated from leaves of 10–15 days old pea plants (*Pisum sativum* cv Rondo) as described before [3] and the isolated thylakoids were frozen and stored at −80 °C. The Hill reaction was measured as oxygen evolution with a Clark-type oxygen electrode equipped with a teflon membrane of high sensitivity [3]. All experiments were carried out at 25 °C. Hill reaction rates are expressed as μmol O_2/mg Chl · h.

Results and Discussion

Incubation of isolated chloroplasts in a medium containing formate results in an inhibition of the Hill reaction in the presence of an uncoupler. If no uncoupler is present a stimulation of electron flow appears (Fig. 1). Concentrations of 100 mM formate and higher were found to give a 3-fold stimulation of the basal electron transport rate. As a similar stimulation is observed with acetate (data not

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Abbreviations: Bicarbonate*, $CO_2 + H_2CO_3 + HCO_3^-$; Chl, Chlorophyll; FeCy, potassium ferrihexacyanate; K'_r, apparent reactivation constant; K_r, reactivation constant; K_i, inhibition constant; PD, *p*-phenylene diamine; PQ, plastoquinone; PS II, photosystem II; Q_A, primary quinone acceptor of photosystem II; Q_B, secondary quinone acceptor of photosystem II; Q_B-protein, 32-kilodalton Q_B and herbicide binding protein; V_{Hill}, Hill reaction rate; V_{max}, maximal Hill reaction rate.

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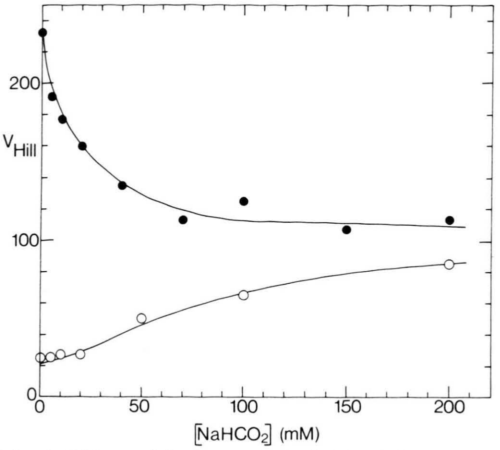

Fig. 1. Effects of formate on the initial Hill reaction rate in chloroplasts in the absence (○) and in the presence of 5 mM NH_4Cl (●) after 1 min dark-incubation in reaction medium. Reaction medium: 0.33 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 50 mM HEPES/NaOH (pH 7.0), 0.5 mM FeCy and chloroplasts equivalent to 25 µg Chl/ml.

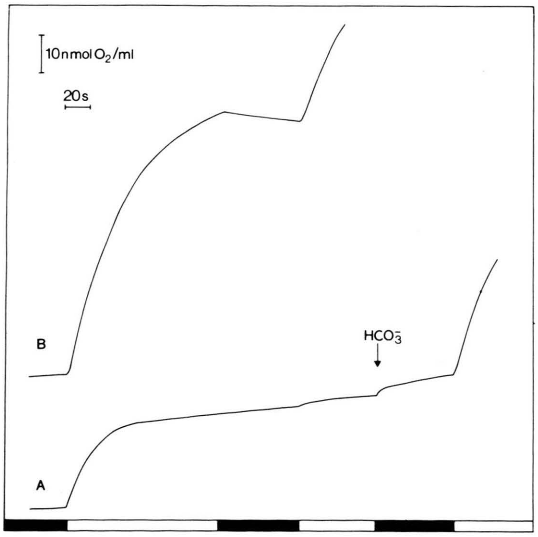

Fig. 2. Effect of bicarbonate* on the time-course of the Hill reaction in the presence of formate. Reaction medium: 0.3 M sorbitol, 100 mM NaCl, 100 mM Na-formate, 50 mM Na-phosphate (pH 6.5), 5 mM NH_4Cl, 1 mM FeCy and chloroplasts equivalent to 25 µg Chl/ml. A: Hill reaction in the absence of added bicarbonate*; B: Hill reaction in the presence of 10 mM bicarbonate*.

shown), we suggest that this stimulation of electron flow is a consequence of the uncoupling effect of anions [12, 13].

The uncoupled Hill reaction appears to be inhibited by formate and acetate, but not by citrate. The Hill reaction rates in Fig. 1 were calculated from the change in oxygen concentration during the first 10 s of the illumination period. Especially at high formate concentrations or at low pH the Hill reaction rate rapidly declines during illumination. The decay appears to be a process with (pseudo) first order kinetics with a half-time which is dependent on *e.g.* formate concentration, pH, surface potential [14] and biotype [15]. Fig. 2A shows the time-course of the Hill reaction of uncoupled chloroplasts in the presence of 100 mM formate. During illumination the Hill reaction declines to a very low value. In a subsequent dark interval the Hill reaction is not reactivated; only after incubation with bicarbonate* in the dark the Hill reaction can be reactivated. If bicarbonate* is added at the beginning of the experiment (Fig. 2B) the Hill reaction rate also declines during illumination, but the inhibition appears to be less severe. In this case only a dark interval is sufficient to restore the original Hill reaction rate. These results are comparable with data given by Good [1] and by Stemler [16] and demonstrate that the reactivation of the Hill reaction in the presence of formate is dependent on the presence of bicarbonate*. Fig. 3 shows the reactivation of the (initial) Hill reaction by various bicarbonate* concentrations in chloroplasts that were inactivated in the light in the presence of formate as shown in Fig. 2A. From the Lineweaver-

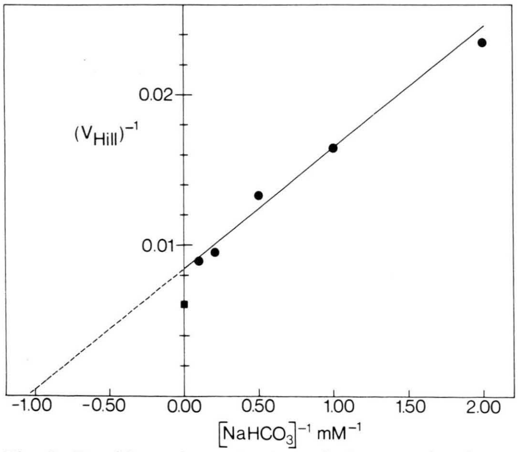

Fig. 3. Double-reciprocal plot of the reactivation of the Hill reaction by various bicarbonate* concentrations in the presence of formate. Reaction medium as in Fig. 2, with the exception that 0.25 mM PD_{ox} was added as an electron mediator, which accepts electrons from PS II. (●), chloroplasts that were inactivated in the light (as shown in Fig. 2A) in the absence of bicarbonate* before bicarbonate* was added; (■), control chloroplasts in reaction medium + 10 mM bicarbonate*.

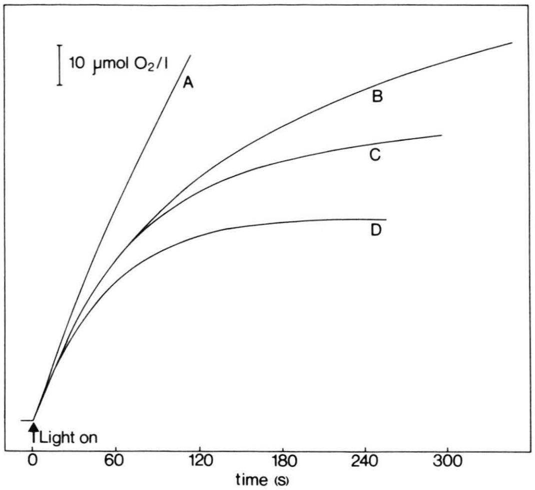

Fig. 4. Effect of bicarbonate* on the time-course of the Hill reaction in the absence and in the presence of 100 mM formate during prolonged illumination. Reaction medium: 0.3 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 50 mM MES/NaOH (pH 6.5), 1 mM FeCy and chloroplasts equivalent to 25 µg Chl/ml. A: absence of formate and bicarbonate*; B: + 100 mM formate, + 20 mM bicarbonate*; C: + 100 mM formate, + 5 mM bicarbonate*; D: + 100 mM formate, + 1 mM bicarbonate*.

Burk plot we calculated an apparent reactivation constant (K_r') of 0.95 mM $NaHCO_3$, which is about the same as reported before [10, 11, 17]. As in the latter experiment chloroplasts were used that were inactivated by incubation with formate at low pH in the dark, the method of deactivation does not seem to affect the reactivation of the Hill reaction by bicarbonate*. The main difference between the two methods is a much lower rate of electron flow when chloroplasts are treated at low pH in the dark. The low pH treatment yielded rates of fully reactivated Hill reaction of at most 50 µmol O_2/mg Chl · h, while at pH 6.5, treatment in the light, rates can be obtained of 120 µmol O_2/mg Chl · h.

We have measured the inhibition of the Hill reaction by formate at several bicarbonate* concentrations in order to see if and at what concentration bicarbonate* is able to prevent the inhibition of electron flow by formate during prolonged illumination. Preliminary results (Fig. 4) show that even in the presence of 20 mM bicarbonate* the reactivation of the Hill reaction is less than 25%. Therefore we assumed that the apparent reactivation constant (K_r' = [bicarbonate*] at 50% reactivation) is greater than 20 mM bicarbonate* in the presence of 100 mM formate during illumination. Under similar conditions K_r' appears to be 1–3 mM bicarbonate* in the dark [10, 11, 17], and therefore we conclude that K_r' is about an order of magnitude higher in the light than in the dark. The value of K_r' is dependent on the formate concentration [11] and is about 0.15 mM bicarbonate* in the presence of 2 mM formate in darkness. If, under these conditions, K_r' is also an order of magnitude higher after prolonged illumination, then K_r' would be about 1.5 mM in the light or, alternatively, less than 2 mM formate would be needed to cause 50% inhibition in the presence of 0.15 mM bicarbonate*.

These simple calculations merely indicate that linear electron flow might be inhibited significantly by formate concentrations less than 1 mM during prolonged illumination at physiological bicarbonate* concentrations.

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