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Effects of Mannose on Photosynthetic Gas Exchange in Spinach Leaf Discs[1]

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GARY C. HARRIS, JOHN K. CHEESBROUGH, AND DAVID A. WALKER
Biology Department, Wellesley College, Wellesley, Massachusetts 02181 (G. C. H.); and A.R.C. Research Group on Photosynthesis, Department of Botany, University of Sheffield, Sheffield S10 2TN England (J. K. C., D. A. W.)

ABSTRACT

When mannose is provided in the transpiration stream to spinach (*Spinacia oleracea*) leaf discs, a series of specific and nonspecific changes occur in CO_2 and H_2O vapor exchange as a function of feeding time. The initial increases in apparent photosynthesis and transpiration are nonspecific effects due to osmotic changes leading to passive stomatal opening. The mannose-specific effects are: (a) time-dependent changes in the CO_2 concentration required for saturation; (b) complex kinetics of the inhibition of CO_2 assimilation dependent on CO_2 and O_2 concentrations and the duration of feeding (high CO_2 and low O_2 lead to rapid inhibitions of photosynthesis); (c) elimination of the capacity of 2% O_2 to stimulate photosynthesis; and (d) oscillations in the CO_2 exchange rate following transitions from 20% to 2% O_2. The mannose-specific effects are reversible by orthophosphate. The mannose-dependent changes in gas exchange are attributed to altered |ATP|/|ADP| ratios.

Previous work has established that when mannose is exogenously supplied to leaf discs it can lead to a progressive decline in the photosynthetic capacity of the tissue. This decline and the accompanying metabolic disturbances (10) have been attributed to the capacity of mannose to sequester Pi (4, 11, 12). In an effort to ascertain the early kinetics of the mannose-induced photosynthetic decline, and also the nature of the changes in the capacity of the leaf to respond to environmental parameters (O_2, CO_2), the photosynthetic gas exchange of spinach leaf discs was monitored during mannose feeding.

MATERIALS AND METHODS

Measurements of CO_2 and H_2O vapor exchange were as reported previously (7).

RESULTS

When mannose was supplied to the transpiration stream of spinach leaf discs at mM concentrations (20–100 mM), several characteristic changes in the H_2O vapor and CO_2 exchange occurred. Leaf temperature dropped and transpiration and apparent photosynthesis increased within minutes of the start of feeding (Fig. 1). These effects were not specific to mannose, however. Their magnitude was greater when high concentrations of compounds which do not penetrate the plasmalemma (*i.e.* potassium ferrocyanide) were used instead of mannose and when the upper limits of the transpiration rate had not already been approached (data not shown). Following these initial nonspecific effects, a series of secondary changes occurred; these were only brought about by sugars which are known to sequester Pi (*e.g.* mannose and 2-deoxyglucose).

When spinach leaf discs were enclosed within the Plexiglas chamber utilized in these experiments, they retained the characteristic C3 photosynthetic responses to changes in external partial pressures of O_2 (2, 7). In saturating light at 25°C and with atmospheric concentrations of CO_2, the change from 20% O_2 to 2% O_2 increased apparent photosynthesis by 45% and lowered the mesophyll resistance (r_m) from 3.8 to 2.2 s/cm (7). The kinetics of the transition to the higher rate under 2% O_2 were without evident oscillations in the rate of CO_2 exchange, and a new steady rate was generally reached in 2 to 5 min (Fig. 2).

Following feeding with either deoxyglucose or mannose, there was progressive decline in the capacity of 2% O_2 to stimulate photosynthesis, leading ultimately to a condition in which low O_2 no longer stimulated photosynthesis and could even become inhibitory (Figs. 1, 2, and 4). This inhibitory effect of low O_2 could be partially relieved by lowering the ambient CO_2 (Fig. 5) or by providing a pulse of Pi (Fig. 3). When an inhibition of apparent photosynthesis by 2% O_2 occurred, it was most evident immediately after the O_2 transition and was sometimes followed by a slow recovery in the rate of CO_2 exchange (Fig. 4a). The elimination of the effect of O_2 on photosynthetic gas exchange was sometimes (Fig. 1) but not always (Fig. 2) accompanied by an overall depression in the rate of gas exchange. The leaf retained the postillumination burst, a compensation point between 40 and 100 μl/l and a CO_2 efflux into CO_2-free air equivalent to 1.3 mg CO_2/$dm^2 \cdot h$. If mannose-treated leaves were maintained at temperatures less than 23°C, the transition from 20% to 2% O_2 resulted in a series of dampening oscillations in the rate of apparent CO_2 exchange (Figs. 1, 2, and 4a). These oscillations had periods of 1 to 2 min and were generally totally damped within 4 to 8 min. The influence of mannose and deoxyglucose on gas exchange was not duplicated by glucose or sorbitol (osmotic stress control) and could not be washed out with water (Fig. 3). There was no evident change in stomatal resistance (r_w) in response to the O_2 transitions (Fig. 1). Under high internal partial pressures of CO_2 (950 μl/l), a transition from 20% O_2 to 2% O_2 led to an initial depression in the rate of assimilation (Fig. 4b). This O_2 transition was followed by a series of dampening oscillations while the rate slowly recovered. The magnitude of the depression of photosynthesis by low O_2 and the amplitude of the oscillations were increased by a prior period of photosynthesis under low CO_2 (350 μl/l) (data not shown).

The progressive decreases in the responses to low O_2 were accompanied by changes in the CO_2 concentration required for saturation, such that atmospheric and subatmospheric levels of

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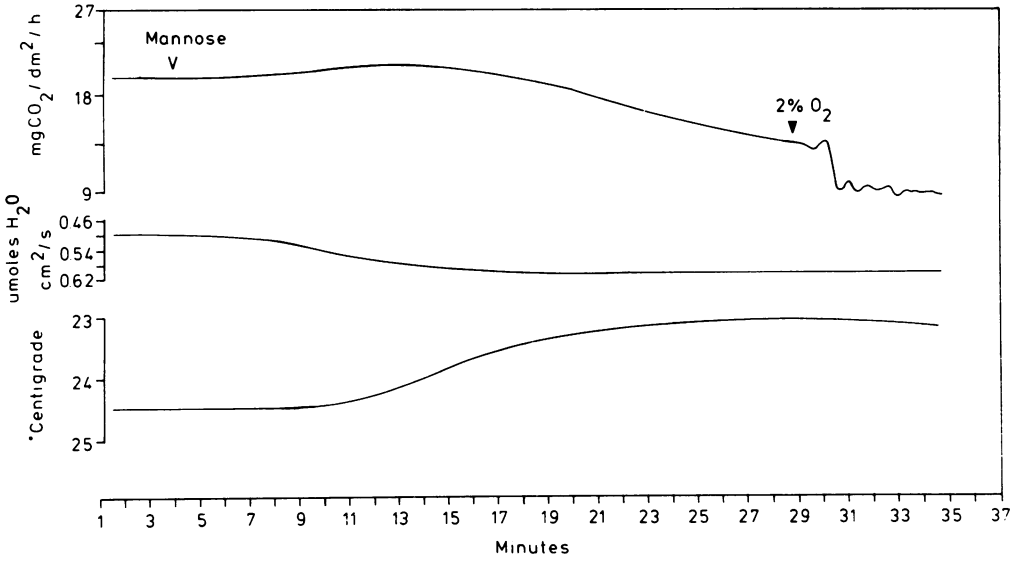

FIG. 1. Effects of mannose (50 mM) feeding on apparent photosynthesis, transpiration rate, and leaf temperature. Note anomalous response to the transition from 20% to 2% O_2 following prolonged mannose feeding.

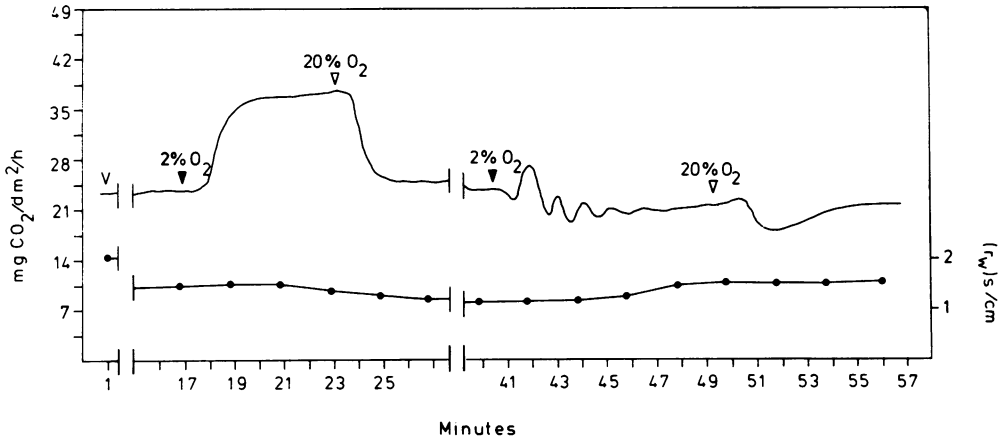

FIG. 2. Effects of continuous mannose (10 mM) feeding (V) on the kinetics of CO_2 exchange during the transitions from 20% to 2% O_2. Apparent photosynthesis (——); resistance to H_2O transfer (●——●).

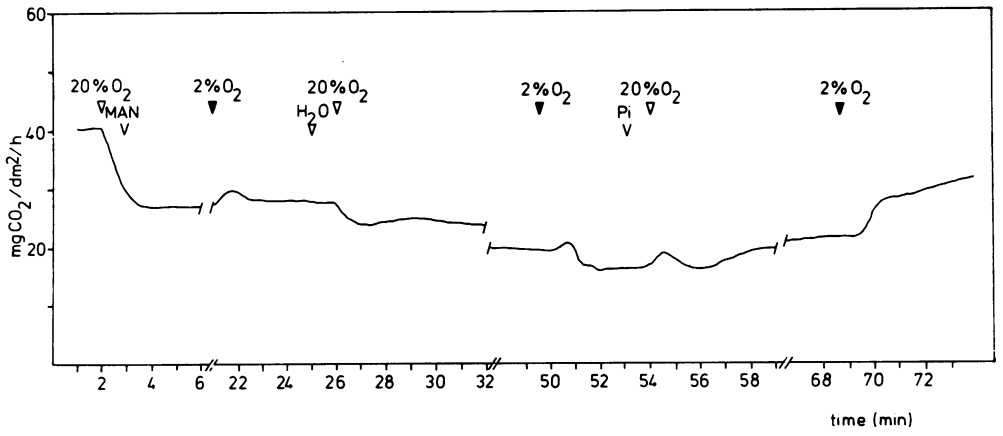

FIG. 3. Capacity of H_2O and Pi (20 mM) feeding to reverse the mannose (20 mM)-induced low O_2 response anomalies.

CO_2 soon become saturating. The results shown in Figure 5, which are manifestations of these saturation phenomena, show that the capacity of mannose to inhibit gas exchange is a function of both the ambient CO_2 and O_2 concentrations and the duration of mannose feeding. In general, it was found that with increasing concentrations of CO_2 and low O_2 the inhibitory effects of mannose appeared sooner and the rate of photosynthesis declined more rapidly. This is clearly seen if those experiments with the largest differentials in ambient CO_2 are compared. For example, in Figure 5b the rate of CO_2 exchange by a disc in 332 μl/l CO_2 dropped rapidly while a disc in 80 μl/l CO_2 was unaffected. In 20% O_2 (Fig. 5a), the inhibitory effects were generally similar

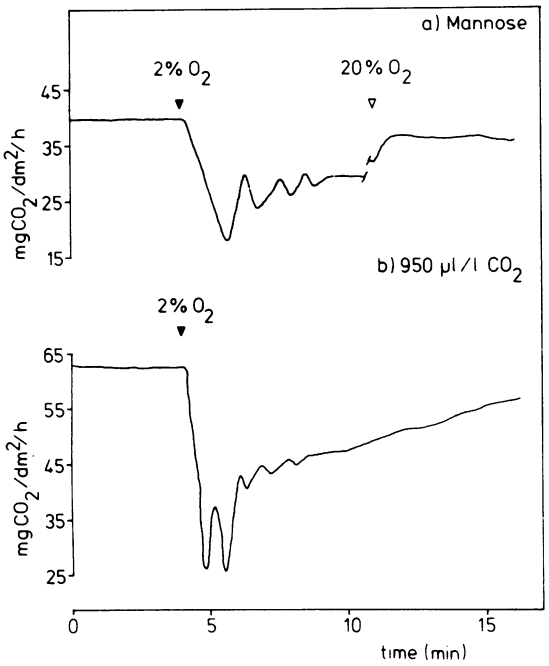

FIG. 4. Effects of (a) mannose feeding (20 mM, 350 µl/l CO_2) and (b) high CO_2 (950 µl/l) on the kinetics of CO_2 exchange during transitions from 20% to 2% O_2.

although they occurred later and were less marked. In the latter stages of mannose feeding, stomatal behavior was partially uncoupled from the photosynthetic response in the sense that significant inhibitions of CO_2 exchange could occur without stomatal response (Fig. 1). This effect could be partly responsible for the dessication noted during prolonged mannose feeding and could be related to the energy requirement for stomatal closure (16).

DISCUSSION

The simultaneous increase in water vapor loss and apparent CO_2 exchange that is seen when mannose is provided in the transpiration stream may be analogous to the 'Iwanoff effect.' The Iwanoff effect is the transpirational surge observed when an actively transpiring leaf is excised from a plant (21). The excision initiates a series of water potential changes that begin in the leaf mesophyll cells and subsequently spread to the overlying epidermis. The decreases in turgor which accompany the water potential changes result in a 'relaxation' of some of the epidermal cells leading to an increase in stomatal apertures (21). Similarly, the addition of compounds such as mannose to the transpiration stream could lead to a rapid extracellular accumulation of that compound and a resultant osmotic loss of water from the mesophyll cells. Osmotic adjustments by cells adjacent to the guard cells could then lead to wider stomatal apertures. The decreasing stomatal resistance (r_w) would allow an increase in the internal concentrations of CO_2 resulting in higher rates of apparent gas exchange.

However, osmotic effects such as these would be transient and could not account for the longer term decreases in resistance to water vapor exchange seen during mannose feeding (9). These long-term changes are more likely to be related to the depletion of endogenous Pi. Hexokinase readily catalyzes the phosphorylation of a variety of hexose analogs such as mannose, and in species like spinach, in which further metabolism of the phosphorylated sugar is not rapid, mannose phosphate accumulates (4; for reviews, see 11 and 12). Accordingly, cytoplasmic Pi pools are depleted (11) leading to inhibitions of O_2 evolution (6) and $^{14}CO_2$ fixation (11), changes in secondary fluorescence kinetics (19), and various

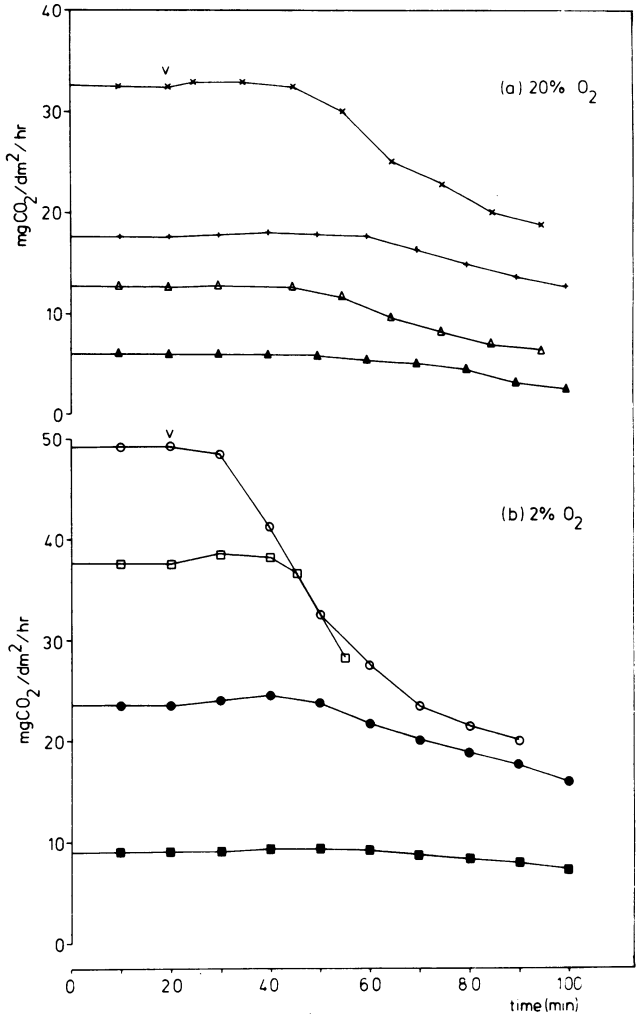

FIG. 5. Effects of mannose (10 mM) feeding on the rate of CO_2 exchange as a function of O_2 ([a], 20% O_2; [b], 2% O_2), CO_2, and time. (×), 516 µl/l; (+), 310 µl/l; (△), 185 µl/l; (▲), 110 µl/l; (○), 732 µl/l; (□), 332 µl/l; (●), 197 µl/l; (■), 80 µl/l.

metabolic changes (4, 10, 11), including a 66% decrease in the tissue [ATP]/[ADP] ratio (unpublished work by authors done in collaboration with M. Stitt and H. Heldt). Additional work by C. Foyer *et al.* (6) utilizing the technique of nuclear magnetic resonance spectroscopy has shown that spinach protoplasts, when isolated from mannose-fed leaf tissue, show increases in the organic phosphate pool that are accompanied by decreases in cytoplasmic Pi. It seems reasonable that the mannose-dependent changes in the gas exchange characteristics be attributable to a transition from CO_2-limited to Pi-limited photosynthesis.

It has been suggested (5, 13, 14) that the assimilation of CO_2 at low internal partial pressures of CO_2 follows the kinetics of RuBP[2] carboxylase-oxygenase. With increasing concentrations of CO_2, however, there is a departure from this relationship, marking the transition from a RuBP-saturated to RuBP-limited rate as the ultimate ceiling imposed by electron transport capacity is approached (14).

The CO_2 partial pressure required for saturation of photosynthesis was progressively decreased during the course of mannose feeding. This is attributed to Pi limitation and the consequent lowered [ATP]/[ADP] ratios which would limit the capacity of the

[2] Abbreviations: RUBP, ribulose-1,5-bisphosphate; PGA, 3-phosphoglycerate.

photosynthetic carbon reduction cycle to supply RuBP (3, 17, 20) and lead, in turn, to decreases in the maximal velocity of photosynthesis.

It should be noted, however, that it is not simply the [ATP]/[ADP] ratio which is important here but that which prevails at a given concentration of PGA. Thus, high PGA can compensate for an otherwise unfavorable [ATP]/[ADP] ratio (17). What is undeniable is that the chloroplast cannot synthesize and export triose phosphate faster than it can import CO_2 and Pi, that the availability of these metabolites is therefore a major factor in the determination of the photosynthetic rates. The rate of photosynthesis in isolated spinach chloroplasts falls to 25% or less of its maximum in the presence of limiting Pi (12, 20).

In the absence of phosphate-sequestering agents and under conditions of limiting CO_2, 21% O_2, and saturating light, carbon assimilation in spinach can be markedly stimulated by a transition to 2% O_2 (2, 7, 13). The inhibition of photosynthesis by 21% O_2 is caused by competition between CO_2 and O_2 in the RuBP carboxylase-oxygenase reaction and by subsequent loss of carbon during glycolate metabolism (2).

It was also found that under conditions of Pi limitation the CO_2 assimilation rate was no longer stimulated and was often inhibited by a transition to 2% O_2. This lack of O_2 sensitivity is not associated with any evident changes in photorespiration (*i.e.* the postillumination burst, the high compensation point, and the CO_2 efflux into CO_2-free air were retained suggesting that the lack of stimulation by low O_2 is not necessarily accompanied by a substantial decrease in photorespiration). The transition from 20% to 2% O_2 in mannose-treated tissues is often accompanied (at leaf temperatures <23°C) by a series of dampening oscillations in the rate of CO_2 exchange which probably mirror similar oscillations in the RuBP pool size. All of the above is consistent with the existence of adenylate-dependent regulatory mechanisms which influence the RuBP supply and/or the kinetics of RuBP carboxylase oxygenase (1, 3, 8, 15, 17, 20).

The possibility that RuBP synthesis is limited in low O_2 has been previously suggested as an explanation of the inhibition of apparent photosynthesis by 2% O_2 under high external CO_2 (RuBP limitation) (1). In the present work, kinetic transients in CO_2 assimilation similar to those found in mannose-fed leaves were observed under conditions of CO_2 saturation. The periodicity of the oscillations seen following the transition to 2% O_2, the inhibition of assimilation by 2% O_2, and the very slow recovery in the rate following a transition to 2% O_2 were all very similar to the mannose-induced response. It may be inferred that the mannose-induced effects are directly related to conditions associated with apparent CO_2 saturation. These are likely to be RuBP limitation coupled with a low [ATP]/[ADP] ratio. Preliminary data with mannose-fed tissue indicate that the lack of O_2 sensitivity at a given partial pressure of CO_2 is nearly coincident in time with that partial pressure becoming saturating for apparent photosynthesis.

A working model based on the known effects of altered adenine nucleotide ratios (3, 17) and O_2 concentrations (2, 13) could account for some of the O_2 response anomalies. Even under RuBP limitation, the transition to 2% O_2 should result in momentary increases in the rate of CO_2 assimilation and PGA formation. This would account for the rate increase seen as the steep upward slope of the first oscillation. However, the increase in assimilation could not be sustained because the pulse of carbon moving through the cycle would further lower an already unfavorable [ATP]/[ADP] ratio. This, in turn, would slow the PGA kinase reaction (17), limiting the regeneration of RuBP and accounting for the downward slope of the oscillation. As the [ATP]/[ADP] ratio improved during the inhibition of CO_2 assimilation, a similar sequence of events would be set in motion. The final steady-state rate of RuBP synthesis would be diminished because of the inability of the system to restore an entirely unfavorable adenine nucleotide ratio under low O_2. Work with the reconstituted chloroplast system has shown that relatively small changes in the [ATP]/[ADP] ratio can profoundly affect photosynthetic O_2 evolution (3).

It is also possible that decreases in P-glycolate synthesis would contribute to this effect. For example, photosynthesis by isolated chloroplasts in high [CO_2] is severely limited in Pi-deficient reaction mixtures (18, 20). However, under conditions which favor P-glycolate synthesis, Pi deficiency might be offset by internal recycling of Pi released in the P-glycolate phosphatase reaction (18). However, it should be noted that the significance of this photorespiratory recycling of Pi is dependent on a fraction of the glycerate either acting as an end product or being phosphorylated in the cytoplasm (2, 18). Clearly, if the cytoplasmic [Pi] is limiting photosynthesis (as it would be in the presence of a mannose-induced Pi deficiency), suppression of P-glycolate synthesis in low [O_2] could exacerbate the limitation and contribute to the observed inhibition by lowered [O_2].

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