Journal of Experimental Botany, Vol. 37, No. 181, pp. 1170–1179, August 1986

Regeneration of Ribulose 1,5-bisphosphate and Ribulose 1,5-bisphosphate carboxylase/oxygenase Activity Associated with Lack of Oxygen Inhibition of Photosynthesis at Low Temperature

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Received 29 January 1986

ABSTRACT

Schnyder, H., Mächler, F. and Nösberger, J. 1986. Regeneration of ribulose 1,5-bisphosphate and ribulose 1,5-bisphosphate carboxylase/oxygenase activity associated with lack of oxygen inhibition of photosynthesis at low temperature.—J. exp. Bot. 37: 1170–1179.

The nature of the lack of oxygen inhibition of C_3-photosynthesis at low temperature was investigated in white clover (*Trifolium repens* L.). Detached leaves were brought to steady-state photosynthesis in air (34 Pa $p(CO_2)$, 21 kPa $p(O_2)$, balance N_2) at temperatures of 20 °C and 8 °C, respectively. Net photosynthesis, ribulose 1,5-bisphosphate (RuBP) and ATP contents, and ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBPCO) activities were followed before and after changing to 2·0 kPa $p(O_2)$.

At 20 °C, lowering $p(O_2)$ increased net photosynthesis by 37%. This increase corresponded closely with the increase expected from the effect on the kinetic properties of RuBPCO. Conversely, at 8 °C net photosynthesis rapidly decreased following a decrease in $p(O_2)$ and then increased again reaching a steady-state level which was only 7% higher than at 21 kPa $p(O_2)$. The steady-state rates of RuBP and associated ATP consumption were both estimated to have decreased. ATP and RuBP contents decreased by 18% and 33% respectively, immediately after the change in $p(O_2)$, suggesting that RuBP regeneration was reduced at low $p(O_2)$ due to reduced photophosphorylation. Subsequently, RuBP content increased again. Steady-state RuBP content at 2·0 kPa $p(O_2)$ was 24% higher than at 21 kPa $p(O_2)$. RuBPCO activity decreased by 22%, indicating control of steady-state RuBP consumption by RuBPCO activity.

It is suggested that lack of oxygen inhibition of photosynthesis at low temperature is due to decreased photophosphorylation at low temperature and low $p(O_2)$. This may be due to assimilate accumulation within the chloroplasts. Decreased photophosphorylation seems to decrease RuBP synthesis and RuBPCO activity, possibly due to an acidification of the chloroplast stroma.

Key words—Oxygen inhibition, photosynthesis, ribulose bisphosphate carboxylase/oxygenase.

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INTRODUCTION

Photosynthesis in soybean leaves and cells is inhibited by oxygen in the manner predicted from the kinetic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Laing, Ogren, and Hageman, 1974; Servaites and Ogren, 1978). Oxygen competitively inhibits the carboxylation and promotes the oxygenation of RuBP (Ogren and Bowes, 1971; Bowes, Ogren, and Hageman, 1971). The biochemical model of C_3-photosynthesis proposed by Farquhar, von Caemmerer, and Berry (1981) integrates the kinetic properties of RuBPCO with parameters describing photosynthetic electron transport capacity to predict rates of carboxylation, oxygenation and regeneration of RuBP *in vivo*.

However, there are physiological conditions under which net photosynthesis is not affected by oxygen as would be expected from the kinetic properties of RuBPCO. Lack of oxygen inhibition or even oxygen enhancement of photosynthesis was observed at high light intensity, low temperature, and normal CO_2 partial pressure in wheat leaves (Jolliffe and Tregunna, 1968), white clover (Mächler and Nösberger, 1978), white mustard, tobacco, and tomato (Cornic and Louason, 1979). The temperature dependence of oxygen inhibition of net photosynthesis in wheat leaf segments at normal or slightly increased $p(CO_2)$ was stronger than expected from RuBPCO kinetics (Arrabaca, Keys, and Whittingham, 1981). McVetty and Canvin (1981) found decreases in net photosynthesis after lowering $p(O_2)$ to 2·0 kPa at increased $p(CO_2)$. These decreases were not due to effects of oxygen on stomatal resistance to CO_2 diffusion.

Assuming an absence of effect on CO_2 translocation, these deviations of C_3-photosynthesis from RuBPCO kinetics have to be explained by effects of low $p(O_2)$ either on the activity of RuBPCO or on events involved in the regeneration of RuBP. McVetty and Canvin (1981) suggested that pseudocyclic photophosphorylation may be decreased at low $p(O_2)$. Pseudocyclic phosphorylation due to photoreduction of oxygen has been shown to be necessary for balancing ATP and NADPH requirements in mesophyll chloroplasts of C_4-plants (Furbank, Badger, and Osmond, 1983). No pseudocyclic electron flow can occur in the absence of oxygen. In *Chroomonas* this seemed to result in over-reduction of electron carriers and an inhibition of cyclic electron flow (Suzuki and Tomoyoshi, 1984*a*, *b*). Inhibition of cyclic and pseudocyclic phosphorylation in the absence of oxygen could be a reason for ATP deficiency and decreased CO_2 fixation (Heber, Egneus, Hanck, Jensen, and Köster, 1978).

However, Sharkey (1985) found that a lack of O_2-inhibition of photosynthesis was always associated with a failure of increased CO_2 pressure to stimulate photosynthesis to the expected degree. Our data confirm this for the lack of O_2-inhibition at low temperature (Schnyder, 1984), and support an alternative explanation which is related to the concentration of inorganic phosphate (P_i) in the chloroplast stroma (Sharkey, 1985). Photophosphorylation may be limited by P_i when organic phosphate levels increase due to an inbalance between CO_2 assimilation and assimilate export at high light intensity, low temperature, high $p(CO_2)$ and low $p(O_2)$.

On the other hand, Schnyder, Mächler, and Nösberger (1984) found that decreased oxygen inhibition at low temperature was associated with partial deactivation of RuBPCO at low $p(O_2)$.

The following experiment was designed to shed light on this oxygen effect. Leaves of white clover (*Trifolium repens* L.) were pre-adapted in air to temperatures at which photosynthesis was expected to respond to low $p(O_2)$ either according to RuBPCO kinetics (20 °C) or deviating from RuBPCO kinetics (8 °C). After steady-state photosynthesis was attained, the $p(O_2)$ was decreased from 21 to 2·0 kPa to study the time course of net photosynthesis, RuBP and ATP contents and RuBPCO activity.

MATERIALS AND METHODS

Plant material

White clover plants (*Trifolium repens* L., ecotype Chur) were propagated vegetatively and grown in pots (15 cm diameter) filled with perlite. The pots were placed in growth chambers (PGV-36, Conviron) at day/night temperatures of 20/16 °C. Relative humidity was 75/80% (day/night). The photoperiod was 16 h with light being provided by a bank of fluorescent tubes and incandescent bulbs giving an irradiance of 400 μmol quanta m^{-2} s^{-1} (400–700 nm) at plant height. Plants were irrigated daily with nutrient solution (Hammer, Tibbits, Langhans, and McFarlane, 1978). The leaves used in the experiments had reached full expansion 6–12 d before sampling.

Gas exchange measurements

CO_2 exchange was measured using an open infrared gas analysis system. One leaf was detached during the photoperiod and placed in a temperature controlled cuvette of brass and perspex having a volume of 12 cm^3. Light was provided by a 400 W sodium vapour lamp separated from the cuvette by an 8·0 cm layer of water. Light intensity at the leaf surface was 430 μmol quanta m^{-2} s^{-1} (400–700 nm). Temperature was either 20 °C or 8 °C. The leaf was pre-adapted in air (21 kPa $p(O_2)$, 34 Pa $p(CO_2)$, balance N_2) until steady-state photosynthesis was established (about 30 min). Then a gas mixture with 2·0 kPa $p(O_2)$ and 34 Pa $p(CO_2)$ prepared by Wösthoff pumps was forced through the cuvette. Gas flow was 1000 cm^3 min^{-1}. The decrease in CO_2 concentration due to photosynthesis was monitored by an IRGA Binos 1 (Leybold-Heraeus) used in the differential mode.

Determination of ATP and RuBP content

Excised leaves were pre-adapted as indicated for gas exchange measurements using a cuvette of brass and glass with a volume of 250 cm^3 and containing a fan. Leaves were rapidly removed from the cuvette after different lengths of time at 2·0 kPa $p(O_2)$, frozen in liquid nitrogen and extracted as described by Perchorowicz and Jensen (1983). Chlorophyll was estimated according to Bruinsma (1963). ATP was measured by the luciferase method using the luminometer and assay chemicals from LKB Wallac (Turku, Finland). RuBP was measured according to the method of Latzko and Gibbs (1974) using RuBPCO prepared according to Mächler and Nösberger (1984).

Determination of the activation state of RuBPCO

Excised leaves were pre-adapted as described for ATP and RuBP determination, rapidly removed from the cuvette, and RuBPCO rapidly extracted and tested as described earlier (Mächler and Nösberger, 1980).

RESULTS AND DISCUSSION

Net photosynthesis

The response of net photosynthesis to a change in $p(O_2)$ from 21 to 2·0 kPa was affected by temperature (Fig. 1). Net photosynthesis increased by about 37% at 20 °C, whereas at 8 °C it increased by only 7%. Net photosynthesis at 8 °C showed a complex oscillating response to the decrease in $p(O_2)$. A slight initial increase was followed by a strong decrease, a second increase, and a second decrease. Then net photosynthesis increased slowly attaining a new steady-state after 12–15 min. A similar oscillating time course of net photosynthesis following a change in oxygen partial pressure from 21 to 2·0 kPa was found by McVetty and Canvin (1981) at high CO_2 concentration.

RuBP consumption

The consumption of RuBP is due in part both to photosynthetic CO_2 fixation and to photorespiratory oxygen fixation. RuBP consumption can be calculated from net photosynthesis (F), the partial pressures of O_2 (O) and CO_2 (C) and the substrate specificity factor of RuBPCO (S). S has been determined for white clover by Lehnherr, Mächler, and Nösberger

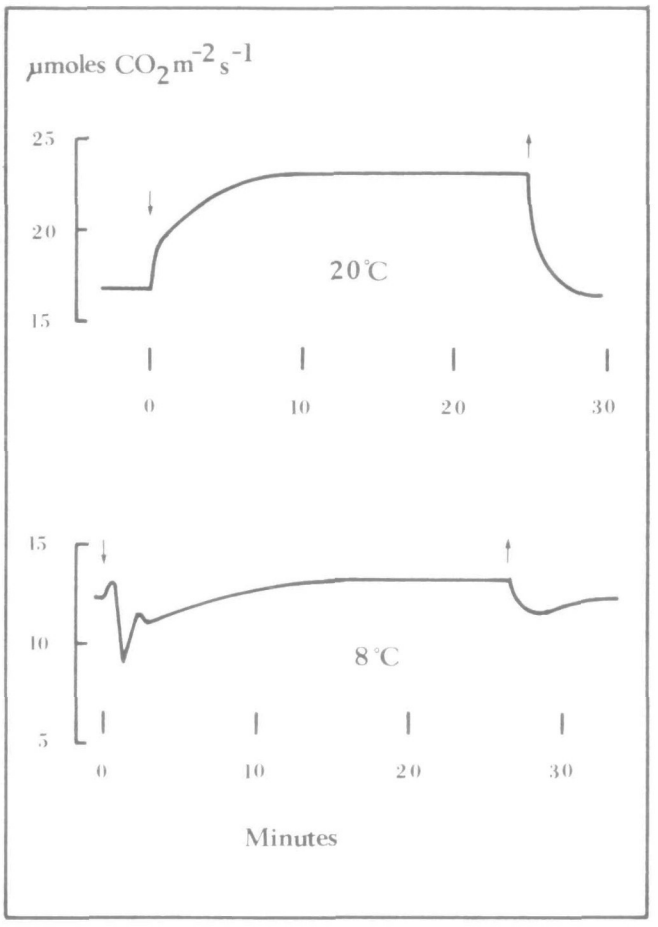

FIG. 1. Time-course of net photosynthesis during a change in O_2 partial pressure from 21 to 2·0 to 21 kPa at 20 °C and 8 °C. Curves represent typical recorder tracing. Changes in oxygen partial pressure are indicated by arrows.

(1985) and is 75 independent of temperature. F and S are related to the velocities of carboxylation (v_c) and oxygenation (v_o) of RuBP by equations 1 and 2.

$$F = v_c - \tfrac{1}{2}v_o \tag{1}$$

$$S = v_c/v_o \cdot O/C. \tag{2}$$

In equation 1 oxygenation of RuBP is related to photorespiratory CO_2 release. Fixation of one molecule of O_2 is followed by the release of half a molecule of CO_2 due to glycine decarboxylation in the glycolate pathway (Ogren and Chollet, 1982). Dark respiration is ignored. Equation 2 is according to Laing *et al.* (1974) and Jordan and Ogren (1981). RuBP consumption ($v_c + v_o$) can be calculated by equation 3, which follows from equation 1 and 2:

$$v_c + v_o = \frac{F}{1 - \dfrac{O}{2SC}} + \frac{F}{\dfrac{SC}{O} - \dfrac{1}{2}}. \tag{3}$$

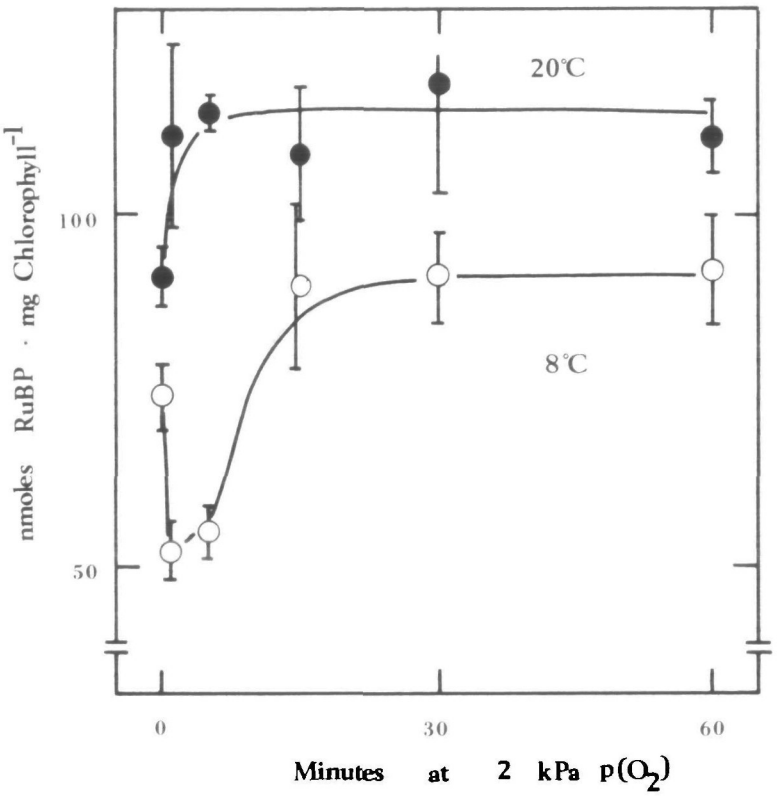

FIG. 2. Tim-course of RuBP content in leaves following a decrease in O_2 partial pressure from 21 to 2·0 kPa at 20 °C (●) and 8 °C (○). Vertical bars indicate 2 × standard errors of means.

The decrease in $p(O_2)$ from 21 to 2·0 kPa was calculated to decrease RuBP consumption per net CO_2 fixed by 31% and 28% at 20 °C and 8 °C respectively. The difference between temperatures is due to a change in the ratio of the solubilities of O_2 to CO_2. The higher efficiency of photosynthetic RuBP consumption at decreased $p(O_2)$ is calculated to allow for a 45% (20 °C) and 39% (8 °C) increase in net CO_2 fixation if RuBP consumption is constant. However, steady-state photosynthesis at 2·0 kPa $p(O_2)$ was only increased by 37% and 7% at 20 °C and 8 °C respectively. Thus steady-state RuBP consumption was calculated to be reduced slightly (−5%) at 20 °C and significantly (−24%) at 8 °C.

RuBP content

The RuBP content in leaves at 20 °C increased by 24% within 1–5 min when oxygen partial pressure was decreased from 21 to 2·0 kPa (Fig. 2). This result is consistent with the findings of Bourquin and Fock (1983). In their experiments, steady-state levels of RuBP were much higher at 2·0 kPa than at 21 kPa $p(O_2)$ if $p(CO_2)$ was low. At high $p(CO_2)$ RuBP levels were similar at low and air level $p(O_2)$. Our results and the data of Bourquin and Fock (1983) suggest that the 24% increase in RuBP content at 2·0 kPa $p(O_2)$ and limiting $p(CO_2)$ found at 20 °C was due to the decrease in RuBP consumption.

At 8 °C, RuBP content decreased immediately by one third after the change in oxygen partial pressure and then increased to attain a steady-state level after 15 min which was 24% higher than the level at 21 kPa $p(O_2)$. The transitional decrease in RuBP content was

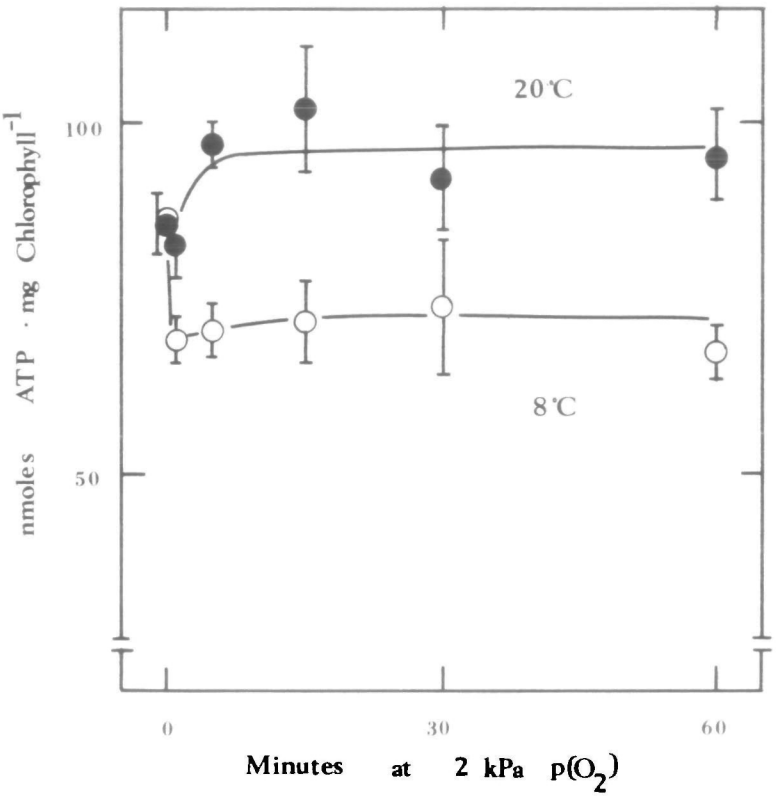

FIG. 3. Time-course of ATP content in leaves following a decrease in O_2 partial pressure from 21 to 2·0 kPa at 20 °C (o) and 8 °C (o). Vertical bars indicate 2 × standard errors of means.

associated with decreased RuBP consumption (see above) indicating reduced RuBP regeneration and limitation of photosynthesis by RuBP.

ATP content

The ATP content in leaves at 20 °C increased by 11% when oxygen partial pressure was decreased from 21 to 2·0 kPa (Fig. 3). At 8 °C it dropped immediately by 18% when oxygen was decreased and did not increase again under steady-state conditions. ATP content as determined in our experiments included a component which was contained in the chloroplast stroma and another component which was contained in other cell compartments. However, it is suggested that oxygen effects on the ATP content as shown in our experiments are due to photosynthesis and reflect changes in the stromal component. Oxygen affects the carbon flow and thus ATP consumption in the Calvin cycle and can affect pseudocyclic and cyclic photophosphorylation. Conversely, ATP synthesis associated with dark respiration in the mitochondria is independent of oxygen above 2·0 kPa $p(O_2)$ (Forrester, Krotkov, and Nelson, 1966).

RuBP regeneration from the products of RuBP oxygenation needs slightly more ATP than RuBP regeneration from the products of RuBP carboxylation. This is due to the need of extra ATP for the phosphorylation of glycerate. Thus the increase in ATP content at 20 °C after a decrease in oxygen (Fig. 3) may be due to a decrease in ATP consumption partly because of an increase in the ratio of carboxylation to oxygenation and partly because of a 7% decrease

in RuBP consumption (see above). The decrease in ATP content at 8 °C following the decrease in $p(O_2)$ was associated with a 24% decrease in RuBP consumption and, due to increased ratio of carboxylation to oxygenation, with an even greater decrease in ATP consumption indicating decreased ATP synthesis. It is suggested that this decrease in ATP synthesis at 8 °C and low $p(O_2)$ caused the transitory decrease in RuBP content (Fig. 2) by decreasing RuBP regeneration.

The decrease in photophosphorylation at decreased $p(O_2)$ could be due to a decreased content of P_i in the chloroplast stroma. Oxygenation of RuBP causes enhanced release of P_i in the chloroplast due to dephosphorylation of phosphoglycolate. This may increase the concentration of P_i in the chloroplast stroma and enhance photophosphorylation (Viil, Ivanova, and Pärnik, 1985). Product inhibition of photosynthesis due to increased levels of organic phosphates and decreased P_i in the chloroplast stroma seems to occur more easily in the absence of oxygen than in its presence since less P_i in the chloroplastic environment is required for assimilate export from chloroplasts if $p(O_2)$ is increased (Usuda and Edwards, 1982). The risk of product inhibition of photosynthesis due to decreased assimilate export from chloroplasts increases as temperature is decreased: A higher P_i level in the chloroplastic environment is required for assimilate export from chloroplasts if temperature is low (Mächler, Schnyder, and Nösberger, 1984). Thus both low temperature and low $p(O_2)$ seem to decrease assimilate export from chloroplasts. Product inhibition of photosynthesis is consequently especially severe when the two effects are superimposed.

Activation state of RuBPCO

The activation state of RuBPCO in leaves at 20 °C was not significantly affected by the decrease in $p(O_2)$ (Fig. 4). Conversely a change from 21 to 2·0 kPa $p(O_2)$ in leaves at 8 °C caused partial deactivation of RuBPCO. After 60 min at 2·0 kPa $p(O_2)$ RuBPCO activity attained a constant level which was 22% lower than at 21 kPa $p(O_2)$.

This decrease in RuBPCO activation seems to be responsible for the low CO_2 fixation rate despite high RuBP content under steady-state conditions at 8 °C and low $p(O_2)$. The initial decrease in RuBP content following the decrease in $p(O_2)$ at 8 °C (Fig. 2) was followed by a 15 min increase. This increase in RuBP content was obviously associated with a slow decrease in RuBPCO activity (Fig. 4) suggesting that initial limitation of photosynthesis by RuBP was relieved by limitation due to decreased RuBPCO activity. However, the decrease in RuBPCO activity appeared to be much slower than the increase in RuBP content. This may be due to the assay procedure for RuBPCO activity. The RuBPCO assay measures the active enzyme-CO_2-Mg^{++} form plus the inactive enzyme-CO_2 form of RuBPCO (Schnyder *et al.*, 1984). The enzyme-CO_2 form may not be present in significant amounts under steady-state conditions (Curry, Pierce, Tolbert, and Orme-Johnson, 1981). However, it may increase when RuBPCO is being inactivated, since the dissociation of CO_2 from the enzyme is much slower than the dissociation of Mg^{++} from the active form. Thus the steady-state equilibrium may be estalished only slowly, especially if temperature is low. Therefore, it is suggested that, after the decrease in $p(O_2)$, RuBPCO activity was overestimated at first in our experiment.

Control of photosynthesis by RuBPCO activity at decreased rates of RuBP regeneration has also been found by Perchorowicz, Raynes, and Jensen (1981) and by Mott, Jensen, O'Leary, and Berry (1984) at low light intensity. A decrease in light intensity was followed by a transitory decrease and then by an increase in RuBP content which was associated with a decrease in RuBPCO activity.

Deactivation of RuBPCO has been found in isolated chloroplasts when assimilate export was decreased due to a low concentration of P_i in the medium (Mächler and Nösberger,

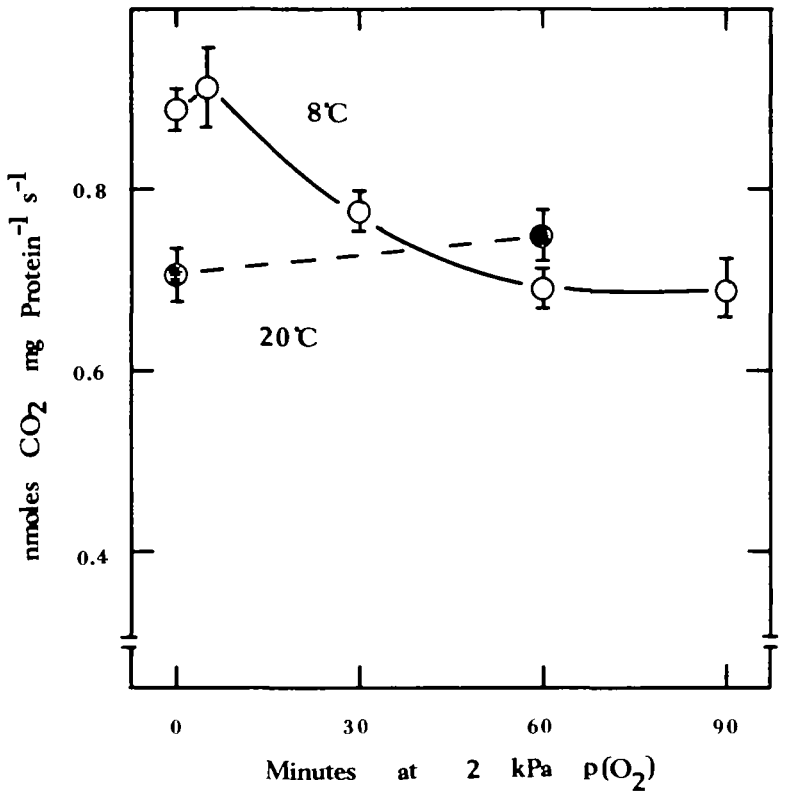

FIG. 4. Time-course of the activation state of RuBPCO in leaves following a decrease in O_2 partial pressure from 21 to 2·0 kPa at 20 °C (o) and 8 °C (o). RuBPCO activity was assayed at 10 °C after rapid extraction. Vertical bars indicate 2 × standard errors of means.

1984). Deactivation of RuBPCO was associated with an accumulation of organic phosphates and a decrease in the ATP content within the chloroplasts and with increased export of 3-phosphoglycerate instead of dihydroxyacetonephosphate (Mächler *et al.*, 1984). Decreased ATP content and preferential export of 3-phosphoglycerate suggested that stromal pH could be decreased and that deactivation of RuBPCO was due to this pH change. A similar mechanism of deactivation of RuBPCO could be suggested for leaves at low $p(O_2)$ and low temperature if it can be assumed that stromal P_i concentration is decreased.

CONCLUSION

Decreased RuBP and ATP consumption, decreased ATP content and initially decreased RuBP content in leaves at 8 °C after a change from 21 to 2·0 kPa $p(O_2)$ suggest a decrease in ATP synthesis and a decrease in RuBP regeneration. The initial limitation of photosynthesis by RuBP was subsequently relieved as RuBPCO activity decreased. It is suggested that the decrease in ATP synthesis was due to decreased stromal concentration of P_i because of assimilate accumulation. This may bring about inactivation of RuBPCO possibly due to a decrease in pH.

ACKNOWLEDGEMENTS

This work was supported by the Swiss National Science Foundation. The authors thank Ms. Allenbach who grew the plants and Ms. Schoenberg who checked the English translation.

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