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# Seasonal Changes in the Kinetic Parameters of a Photosynthetic Fructose-1,6-Bisphosphatase Isolated from *Peltigera rufescens*[1]

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## ABSTRACT

**The kinetic parameters of the photosynthetic fructose-1,6-bisphosphatase isolated from *Peltigera rufescens* (Weis) Mudd. were measured on a seasonal basis and during a laboratory-induced temperature acclimation. Both the substrate affinity and Ea changed on a seasonal basis. During the summer, the Ea decreased from 91.8 to 62.3 kilojoules per mole. The $K_m$ fructose-1,6-bisphosphate measured at temperatures above 25°C was also found to decrease by 50%. This seasonal change in $K_m$ can be induced by growing the lichen under appropriate conditions for 2 weeks, and is correlated to a change in the net photosynthetic rates. It is hypothesized that this change in fructose-1,6-bisphosphatase is related to the seasonal temperature acclimation process that has been previously reported in this species.**

*Peltigera rufescens* is a foliose lichen characteristic of exposed sunny areas. This, in combination with its prostrate growth form and dark thallus coloration, exposes the plant to extremes in temperatures. Maximum thallus temperatures may exceed 50°C during the summer (18). In addition, its evergreen nature allows the lichen to be physiologically active in the winter, at temperatures near freezing. These highly variable, yet seasonally predictable temperature changes suggest that *P. rufescens* could seasonally acclimate its photosynthetic temperature optimum, a photosynthetic strategy which has been previously documented in other lichens and higher plants (5, 13).

Brown and Kershaw (6) have recorded the seasonal pattern of net photosynthesis in this lichen. $CO_2$ exchange rates remain seasonally constant at temperatures of 5, 15, and 25°C; however, maximum rates of net photosynthesis double at 35 and 45°C during the summer months, resulting in a 10°C shift in the temperature optimum of this process. As this temperature acclimation was observed only under saturating levels of illumination, Brown and Kershaw hypothesized that changes in the Calvin-Benson cycle reactions were responsible for the photosynthetic capacity changes (6, 7).

A similar acclimation response, observed in the desert evergreen shrub *Nerium oleander*, was correlated to changes in the activity of FBPase[2] (1), an important regulatory enzyme in the Calvin-Benson cycle. An FBPase with characteristics similar to the photosynthetic enzyme has recently been isolated from this lichen (8). This paper reports on the differences in kinetic properties of this FBPase purified from winter and summer collections of *P. rufescens*, as well as changes in this enzyme extracted from lichens undergoing a laboratory-induced winter acclimation process.

## MATERIALS AND METHODS

**Plant Material.** *Peltigera rufescens* (Weis) Mudd. was collected from rock outcrops in the Port Severn area of Ontario during the winter and summer of 1985, transported back to McMaster University, and stored in an air-dry state in controlled environmental growth chambers. Material collected from December to April was stored under winter conditions of −2°C night/5°C day with a 9 h photoperiod, while those collected from May to September were stored under summer conditions of 25°C night/32°C day with a daylength of 15 h (6). The lichen was allowed to equilibrate to these storage conditions for 1 week prior to any experimentation.

**FBPase Purification and Assay.** Protein was extracted with a 30 mM Pipes buffer (pH 6.6), containing 20 mM 2-mercaptoethanol, 20 mM sodium metabisulfite, and 0.5 g·g dry thallus $wt^{-1}$ of insoluble PVP following the procedure outlined previously (8). Protein content was assayed with the Coomassie blue binding method (Biorad Laboratories) using BSA as a standard.

The photosynthetic FBPase was purified following the method of Buchanan (9) with minor modifications (8). Specific activity of the purified FBPase varied greatly between extractions, ranging from 0.44 to 8.01 nmol·$\mu g^{-1}$ protein $min^{-1}$ at 25°C. FBPase activity was assayed at temperatures between 5 and 45°C by following the change in absorbance at 340 nm in a solution containing 100 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 20 mM DTT, 4 units of phosphoglucose isomerase, and 4 units of glucose-6-P dehydrogenase in a final volume of either 1.0 or 3.0 ml (8). The enzyme kinetic parameters, $K_m$ and $V_{max}$, were calculated from a direct linear plot (10) of a minimum of 15 points using FBP concentrations varied in a 50% dilution series from 0.313 to 0.010 mM. Initial rates were determined by the cord method of Waley (30). Activation energies were calculated from a least square regression of the Arrhenius plots of the calculated $V_{max}$ values and the enzyme rate measured at 0.020 mM FBP.

**Acclimation Time Series.** The time course of temperature acclimation was followed in a collection of *P. rufescens* made during September 1985. Lichen stored under summer conditions of 25°C night/32°C day with a 15 h photoperiod was moved to winter conditions of −2°C night/1°C day and a 9 h photoperiod. This treatment had previously been shown to induce a winter pattern of photosynthetic response (6). The substrate affinity of FBPase was measured at 35°C in the crude extracts.

**Photosynthesis-Irradiance Curves.** Net photosynthetic rate changes during the acclimation process were measured with a Beckman 865 IRGA at 35°C using the discrete sampling method of Larson and Kershaw (15). Apparent quantum efficiency was

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[2] Abbreviations: FBPase, fructose-1,6-bisphosphatase; FBP, fructose-1,6-bisphosphate.

calculated from a least square linear regression of the gas exchange values at light intensities from 0 to 100 $\mu$mol photons $\cdot m^{-2} s^{-1}$. Light saturated rates of net photosynthesis were assayed at 800 $\mu mol \cdot m^{-2} s^{-1}$.

## RESULTS

The relationship between FBPase activity and temperature is complex. The Arrhenius plots are nonlinear, with a distinct break occurring near 25°C (Fig. 1). In addition, there is a significant seasonal trend in the activation energies predicted from this plot. When calculated from $V_{max}$ (representing substrate saturated rates), the Ea in the temperature range of 5 to 20°C was significantly lower in the summer population (Table I). No significant differences were observed between the summer and winter populations for the Ea in the 30 to 45°C range. However, when this parameter was calculated from the activity measured at 0.020 mM FBP the pattern was reversed—no seasonal changes were observed between 5 and 25°C, and the Ea decreased during the summer for temperatures above 25°C.

The substrate affinity of the purified FBPase also showed a strong interaction between seasons (Fig. 2). The enzyme extracted from the winter population had a minimum $K_m$ of 0.028 mM FBP at 5°C. Substrate affinity then decreased as temperature was raised, reaching a maximum $K_m$ of 0.044 mM at 40°C. The summer population had a similar affinity for FBP at the lower assay temperatures. However, at higher temperatures, the $K_m$ for this enzyme decreased, reaching a minimum of 0.019 mM at 45°C. This resulted in a large seasonal difference in the $K_m$ for FBP at temperatures above 25°C (Fig. 2).

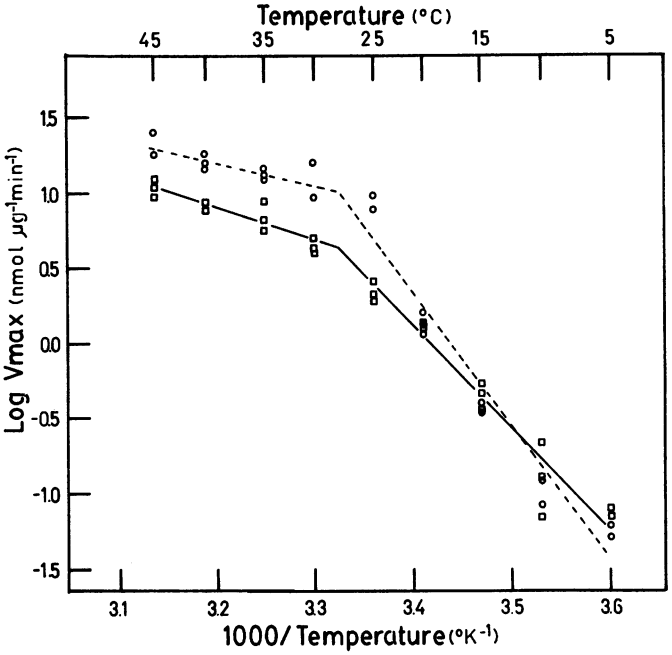


FIG. 1. The Arrhenius plots of the FBPase, purified from a representative summer (□) and winter (○) collection of *P. rufescens*.

Table I. *Seasonal Changes in Activation Energy of the Photosynthetic FBPase Isolated from P. rufescens*

| Season | Temperature | Ea | *t*-test |
|---|---|---|---|
| | | $kJ \cdot mol^{-1} \pm SE$ | |
| Ea calculated from $V_{max}$ | | | |
| Summer | 5–20°C | 93.3 ± 15 | |
| Winter | | 130.0 ± 7.8 | 2.353[a] |
| Summer | 30–45°C | 58.9 ± 11 | |
| Winter | | 82.8 ± 12 | 2.306 |
| Ea calculated at 20 $\mu$M FBP | | | |
| Summer | 5–25°C | 90.4 ± 16 | |
| Winter | | 110.0 ± 5.6 | 1.31 |
| Summer | 30–45°C | 62.3 ± 4.3 | |
| Winter | | 91.8 ± 9.4 | 2.41[a] |

[a] $P > 0.05$, $df = 8$.

This seasonal change in substrate affinity can be mimicked in the laboratory by altering the storage conditions of the lichen (Fig. 3a). Crude extracts from thalli collected in September showed a $K_m$ of 0.026 mM FBP at 35°C, characteristic of the purified FBPase from the summer population. When the plants were shifted to winter storage conditions, substrate affinity remained unchanged for the initial 4 d, and then gradually increased to a $K_m$ in excess of 0.040 mM similar to the $K_m$ of 0.044 mM for the purified winter enzyme. This change in FBP affinity was readily reversible. When the winter stored material was moved back to summer conditions, the $K_m$ rapidly returned to summer levels (Fig. 3a). Specific activity of the FBPase did not change in the crude extract during this induced acclimation; however, there was an increase in the total protein content of the thallus from d 5 through 15 (Fig. 3b). This resulted in a transient increase in total FBPase activity during the period when substrate affinity was changing (Fig. 3c).

The induced change in substrate affinity was paralleled by a change in the light saturated rate of photosynthesis. Gas exchange in the September collection was typical of the summer rates observed previously (6), with a rate of 8.7 mg $CO_2$ $g^{-1}$ $h^{-1}$ at 35°C, 800 $\mu mol \cdot m^{-2} s^{-1}$ (Fig. 4a). This rate gradually declined over a 10 d period while under 'winter' conditions to a value of 6 mg $CO_2$ $g^{-1}$ $h^{-1}$, slightly higher than the December rate of 4.19 mg $CO_2$ $g^{-1}$ $h^{-1}$ measured by Brown and Kershaw (6). As in the case of FBPase affinity, this rate change was readily reversible, increasing to 10.8 $CO_2$ $g^{-1}$ $h^{-1}$ following 3 d of summer storage (Fig. 4a). A similar pattern was observed when the gas exchange rate was based on a Chl content (Fig. 5a), indicating that the change in net photosynthesis cannot be the result of an environmentally induced alteration in the algal or Chl density.

Apparent quantum efficiency, when measured on either a dry weight or Chl basis, was variable, but does not show any definite trends during the experiment (Figs. 4b, 5b).

It is important to emphasize that the changes in net photosynthesis during the induced acclimation experiment were not cor-

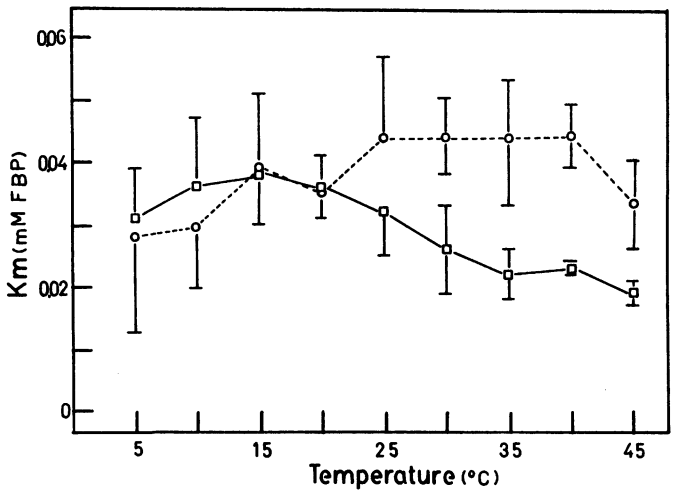


FIG. 2. The relationship between substrate affinity (mean ± SE) and temperature for FBPase isolated from summer (□, $n = 4$) and winter (○, $n = 6$) collections of *P. rufescens*.

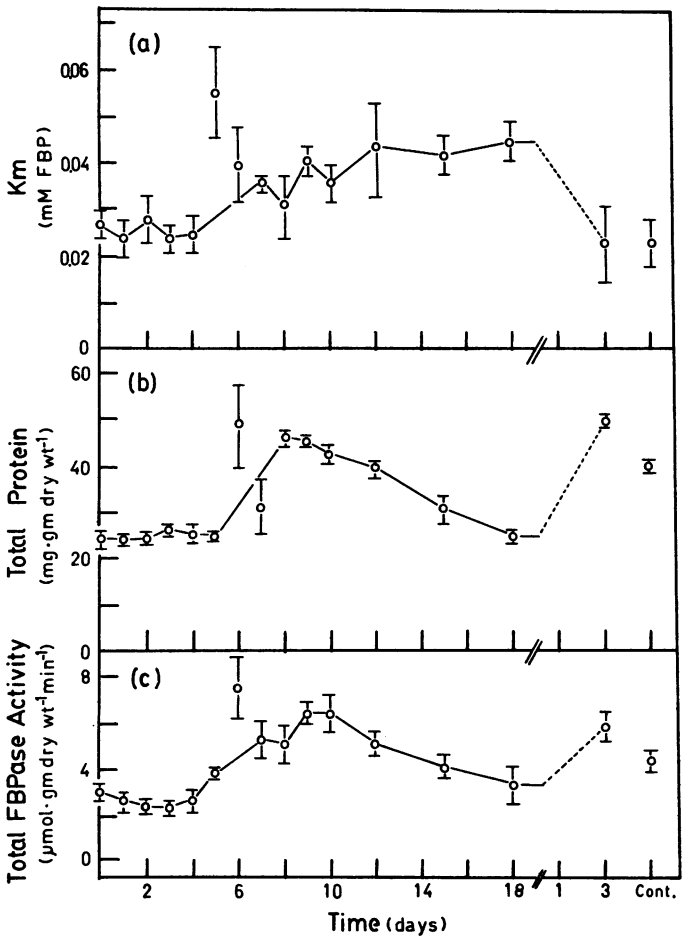


FIG. 3. The change in substrate affinity (a), total protein (b), and total FBPase activity (c) in crude extracts from *P. rufescens* undergoing a laboratory-induced winter acclimation. Lichen collected in September and stored under summer conditions were moved to winter conditions on d 0, and then returned to summer on d 19. Each point represents the mean ± SE of 3 replicate extractions measured at 35°C. Control material maintained under summer conditions was assayed on d 24.

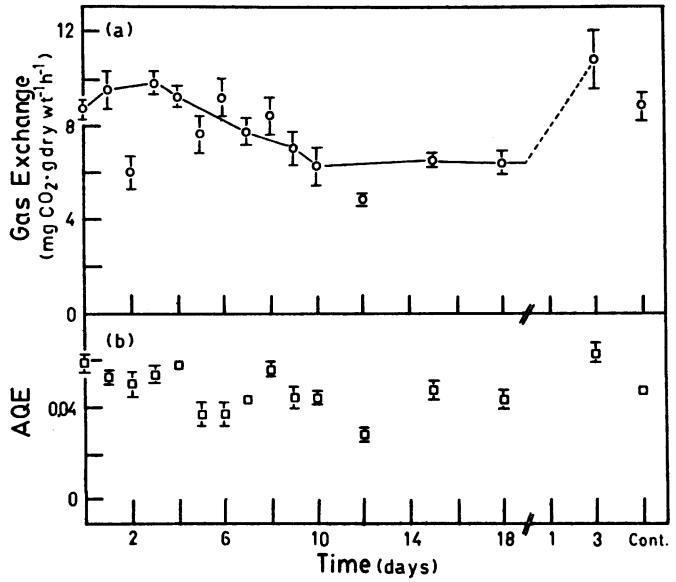


FIG. 4. The change in light saturated (800 $\mu$mol·m$^{-2}$ s$^{-1}$) net photosynthesis (a) and apparent quantum efficiency (AQE, b), assayed at 35°C and calculated on a per thallus dry weight basis, during the acclimation experiment of Figure 3.

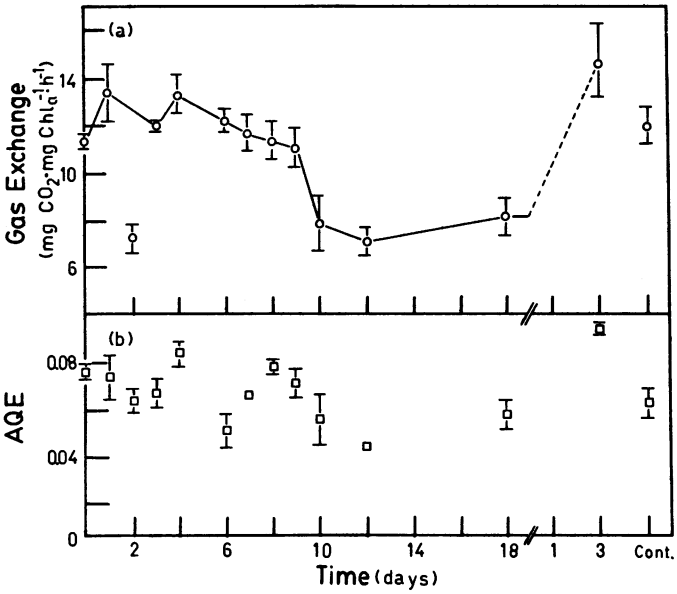


FIG. 5. The change in light saturated (800 $\mu$mol·m$^{-2}$ s$^{-1}$) net photosynthesis (a) and apparent quantum efficiency (AQE, b), assayed at 35°C and calculated on a per mg Chl basis, during the acclimation experiment of Figure 3.

related to changes in total FBPase activity, but only to changes in substrate affinity. The amount of FBPase extracted from the thalli on day 0 is sufficient to maintain a gross photosynthetic rate of 16 mg $CO_2$ g$^{-1}$ h$^{-1}$. When the fungal respiration rate of 3 mg $CO_2$ g$^{-1}$ h$^{-1}$ is taken into consideration, the observed rate of gross $CO_2$ fixation is 12 mg $CO_2$ g$^{-1}$ h$^{-1}$ (Fig. 3a). This indicates that the FBPase *in situ* is operating at 75% of $V_{max}$, which corresponds to a substrate concentration of 0.075 mM FBP. The observed shift in the $K_m$ from 0.025 mM to 0.040 mM FBP (Fig. 3a) would decrease the FBPase activity to 65% of $V_{max}$ assuming all else remained constant. This corresponds to a net gas exchange rate of 7 mg $CO_2$ g$^{-1}$ h$^{-1}$, which is slightly higher than the rates observed at the end of the winter acclimation experiment (Fig. 4a).

The winter acclimation experiment of Figures 3, 4, and 5 suggest some very important ideas, but the interpretation of these results requires a note of caution. Due to the time commitment, it was impractical to purify the FBPase for this experiment. Thus, the changes are measured in a crude extract which may contain several different FBPases (8). Both cytosolic and photosynthetic enzymes are active above pH 8.0, but three observations suggest that the activity of a cytosolic FBPase can be discounted in this experiment. First, the substrate saturation curves of the crude extract followed Michaelis-Menten kinetics. The cytosolic FBPase has a $K_m$ of 0.003 mM FBP (8). If a cytosolic enzyme was active in the crude extract at pH 8.5, the substrate saturation curves would depart from Michaelis kinetics at low concentrations, which was not observed. Second, the $K_m$ changes measured during the acclimation (Fig. 3a) were similar to those observed for the purified FBPase on a seasonal basis (Fig. 2). Third, the FBPase activity in the crude extract was not inhibited by fructose-2,6-bisP an inhibitor of the cytosolic FBPase (8). These three points suggest that the photosynthetic enzyme forms the major part of the activity measured at pH 8.5 in the crude extracts.

## DISCUSSION

Terrestrial plants, especially those growing in exposed habitats, must operate in highly variable temperature regimes, ranging

from the rapid and unpredictable short term fluctuations due to sunfleck activity, to the more predictable diurnal and seasonal patterns. Some plants, notably the short lived annuals and ephemerals, and those with solar tracking abilities, can avoid some of these temperature fluctuations, but many plants are faced with the difficult task of maintaining an internal homeostasis while experiencing wide fluctuations in a major enzymic rate determining factor, temperature.

McNaughton (19) while studying the temperature effects on the enzyme kinetic parameters of malate dehydrogenase isolated from several widely distributed populations of *Typha latifolia*, proposed that plants have evolved a thermal insensitivity to temperature regimes similar to their native environment. Rate effects due to short term temperature fluctuations are partially compensated by a positive relationship between $K_m$ and temperature (27, 28). Thus, as temperature increases, substrate affinity decreases, lessening the temperature dependence of the reactions (4).

Attempts to maintain homeostasis on a longer, seasonal basis can involve both quantitative and qualitative changes. Adaptation to low temperature can be obtained simply through a change in total enzyme activity, as observed in *Laminaria hyperborea* (14), *Nerium oleander* (1), and *Triticum aestivum* (20). No changes in total FBPase activity were observed in *P. rufescens*, either on a seasonal basis (data not shown) or on an experimentally induced basis (Fig. 3c).

Qualitative changes have been observed affecting the substrate affinity, activation energy and thermal stability of the enzyme. A change in activation energy has been considered a major factor in adapting to different thermal environments (19). Observations of different populations of a species have shown that Ea tends to be lower in cooler environments (17, 22, 25). Changes in Ea have not ben reported elsewhere on a seasonal basis, but several studies show that adaptive changes in this parameter can be induced by altering the growth temperature of the plant (23, 24, 26), suggesting that seasonal changes in Ea could be involved in the acclimation process. In *P. rufescens*, the Ea determined from the $V_{max}$ calculations, representative of substrate saturated activity, did change on a seasonal basis at the lower assay temperatures (Table I), but this change appears to be counter adaptive, with Ea increasing from 93.3 to 130 $kJ \cdot mol^{-1}$ for the 5 to 20°C temperature interval during the winter. Thus, the enzyme is less energetically efficient at lower temperatures during the winter. This pattern contradicts the above theory (19), and the change does not appear to be ecologically important as there is no evidence for a decrease in photosynthetic efficiency at low temperatures in the winter (6). As most enzyme systems do not operate at a saturating substrate concentration *in vivo*, an estimate of enzyme efficiency at subsaturating concentrations may be more physiologically realistic (4, 21). When this 'pseudo' activation energy is calculated for a limiting substrate concentration of 0.020 mM FBP, the seasonal pattern was reversed (Table I). The Ea calculated for the 30 to 45°C temperature interval decreased from 91.8 to 62.3 $kJ \cdot mol^{-1}$ during the summer, while no significant changes were observed at the lower temperatures. This pattern correlates with the observed seasonal change in net photosynthesis (6).

This differing response in Ea to substrate concentration has been observed previously, and is the result of a change in the $K_m$-temperature relationship (21), stressing the importance of substrate affinity changes to the acclimation process. Somero (27, 28) has emphasized the need for seasonal adjustments in the temperature response of $K_m$, such that the $K_m$ is minimal at the lower limit of tissue temperature likely to be experienced in the field. This type of seasonal adaptation has been observed in fish, nematodes, and several plant species (2–4, 12, 21, 29).

A similar process seems to be occurring in *P. rufescens* (Fig. 2). In the winter, minimum $K_m$ values occur at 5°C, whereas in the summer, the $K_m$ minimum has shifted to 45°C. This results in a large seasonal change in the substrate affinity at temperatures above 25°C, but there is no difference below this temperature. This correlates well with the changes in net photosynthesis observed seasonally (6), where the photosynthetic capacity above 25°C doubles during the summer, and experimentally, where an increase in $K_m$ is paralleled by a decrease on the gas exchange rates (Figs. 3a, 4a, 5a). As FBPase is considered to be a rate limiting step in the Calvin-Benson cycle (1, 8, 16), changes in the FBPase kinetic properties could be directly related to the observed net photosynthesis patterns.

It is apparent from these data that there is a change in the kinetic form of the FBPase between summer and winter. Badger *et al.* (1) report that photosynthetic temperature acclimation in *Nerium oleander*, which involves a change in the total FBPase activity, occurs over a 2-week period. Although this time course is similar to that observed here (Fig. 4a), only a transient change in FBPase activity was observed (Fig. 3c). It is likely that this transient increase is an integral part of the acclimation process, where both summer and winter forms of the enzyme could be present, but it does not necessarily indicate synthesis of a new enzyme. Huner and Macdowall (12) report that winter hardening in Puma rye involves a similar change in the $K_m$-temperature interaction of ribulose 1,5-bisphosphate carboxylase, that results from a conformational change in the enzyme (11).

Although it requires 2 weeks for a laboratory induced acclimation to produce a stable winter net photosynthetic rate (Figs. 4a, 5a) and change in $K_m$ (Fig. 3a), this process can be quickly reversed. Gas exchange and substrate affinity will return to summer levels within 3 d of transferring the lichen back to summer conditions (Figs. 3a, 4a, 5a; [6]). Total FBPase activity and total protein still remained higher than the characteristic summer level (Fig. 3, b and c), indicating that there may be some metabolic shifts occurring on d 3. It is apparent from this radical difference in the time course, and the previous inability to induce experimentally a summer acclimation in a winter population of *P. rufescens* (6), that warm temperature acclimation is not a strict reversal of the cool acclimation process, and further studies are required to uncover the mechanisms involved.

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