Phytochemistry, Vol. 22, No. 4, pp. 835–840, 1983.
Printed in Great Britain.

0031–9422/83/040835–06$03.00/0

CHARACTERISTICS OF α-GLUCAN PHOSPHORYLASE FROM *CHLORELLA VULGARIS*

YASUNORI NAKAMURA* and MASAKO IMAMURA

Radioisotope Centre, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

(*Revised received* 16 *September* 1982)

Key Word Index—*Chlorella vulgaris*; Chlorophyceae; phosphorylase; starch; ADP-glucose; UDP-glucose.

Abstract—α-Glucan phosphorylase from *Chlorella vulgaris* has been partially purified. In the direction of glucan phosphorolysis the apparent K_m for Pi was *ca* 2.4 mM at pH 7.1. In the direction of glucan synthesis the K_m for G1P was *ca* 0.12 mM at pH 6.2. The enzymic activity was inhibited by physiological concentrations of ADP, ATP, ADPG and UDPG. In the direction of starch degradation in the presence of 2.4 mM Pi the $I_{0.5}$ values for ADP and ATP were *ca* 1.6 and 2.9 mM, respectively, while in the direction of synthesis in the presence of 0.12 mM G1P the values were *ca* 0.23 and 1.4 mM, respectively. The Hill plots for starch degradation showed n values of 2.2 for ADP and 2.2 for ATP and values of 1.5 and 1.2, respectively, for starch synthesis. Both ADPG and UDPG were linear competitive inhibitors either with respect to Pi or with respect to G1P. The K_i values for ADPG and UDPG in the direction of phosphorolysis were shown to be *ca* 0.11 and 0.51 mM, respectively, and those in the direction of synthesis 0.033 and 0.15 mM, respectively.

INTRODUCTION

The regulatory mechanisms of starch metabolism in green plants have not been fully worked out. However, a number of recent studies with chloroplasts isolated from spinach and pea leaves have suggested that α-glucan phosphorylase plays a principal role in the degradation of starch photosynthesized in green plant leaves [1–8]. Nakamura and Miyachi [9, 10] also have proposed that starch phosphorylase participates in the temperature-dependent degradation of starch which had been photosynthesized in *Chlorella vulgaris* 11 h cells.

Several subcellular fractionation studies have shown that phosphorylase is localized in chloroplasts as well as in the cytoplasm both in higher plant leaves [11–13] and the green alga, *Dunaliella marina* [14]. Recently, the properties of chloroplast phosphorylase from various plant leaves have been investigated [13, 15–19]. However, little work has been done on the regulatory characteristics of α-glucan phosphorylase in algae. Therefore, the present paper deals with the kinetic properties and the mode of regulation of the activity of partially purified phosphorylase from *Chlorella vulgaris*. This paper shows that phosphorylase from *Chlorella* is markedly regulated by physiological concentrations of various metabolites, e.g. ATP, ADP, ADPG and UDPG which have been reported to be ineffective towards chloroplast phosphorylase from higher plant leaves [15, 16, 19].

*To whom requests for reprints should be addressed.

Abbreviations: DTT, dithiothreitol; G1P, glucose 1-phosphate; 3-PGA, 3-phosphoglycerate; ADPG, adenosine diphosphate glucose; UDPG, uridine diphosphate glucose; Pi, inorganic phosphate.

RESULTS

DE-52 chromatography of Chlorella *phosphorylase*

Figure 1 shows that *Chlorella* phosphorylase activity was eluted as a single peak at *ca* 0.13 M sodium chloride. This finding is a sharp contrast to previous reports that a number of plant tissues contain multiple forms of α-glucan phosphorylase which can be separated by DEAE chromatography [20, 21]. The recovery of the total activity of the *Chlorella* phosphorylase was *ca* 55%, whereas that of the total protein content was *ca* 40%. Therefore, it is most likely that the peak of the activity accounted for at least, if not exclusively, the bulk of phosphorylase contained in *C. vulgaris* cells.

Initial velocity studies

Lineweaver–Burk plots for the initial rate of α-glucan phosphorolysis by *Chlorella* phosphorylase at varying concentrations of Pi at a series of fixed concentrations of potato amylopectin gave a series of straight lines with a common x-axis intercept (Fig. 2). The K_m value for Pi was estimated to be *ca* 2.4 mM. Double reciprocal plots of initial reaction velocities in the direction of α-glucan synthesis against the reciprocal concentration of G1P also intersected on the x-axis (Fig. 3). The K_m value for G1P was *ca* 0.12 mM.

These primary plots show the characteristics of a rapid equilibrium random Bi–Bi kinetic mechanism which polyglucan phosphorylase from all sources investigated appears to have [22].

The K_m (Pi) for *Chlorella* phosphorylase is in the same range of those for the chloroplast enzyme from higher plant leaves [13, 16, 19]. It should be noted, however, that the K_m (G1P) for *Chlorella* phosphorylase is much lower than that reported for the other plant sources including

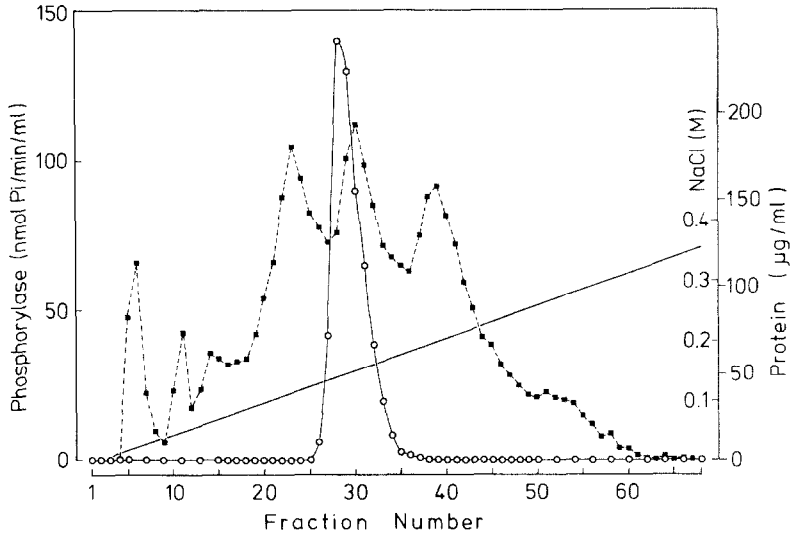

Fig. 1. Elution pattern of phosphorylase on DE-52 chromatography of a dialysed 30–70% ammonium sulphate precipitate prepared from a *C. vulgaris* cell extract. (○) Phosphorylase activity in the direction of starch phosphorolysis; (■) protein content.

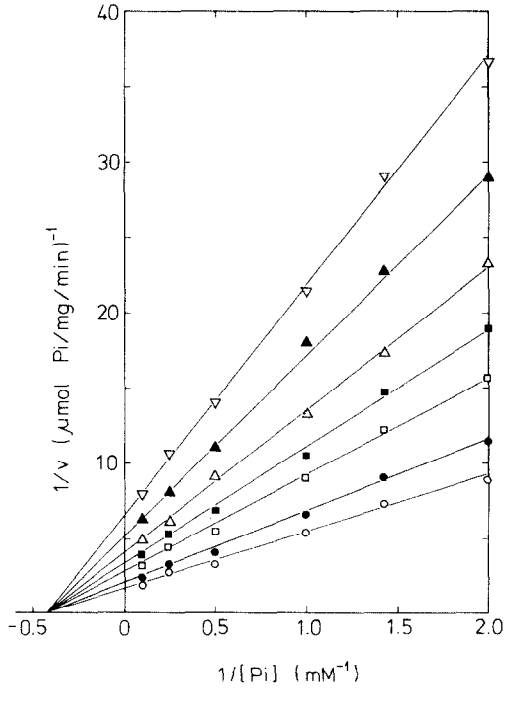

Fig. 2. Lineweaver–Burk plot of initial velocity of *Chlorella* phosphorylase against Pi at a series of fixed concentrations of amylopectin. The amylopectin concentrations were (mg/ml): 4.0 (○); 1.6 (●); 0.8 (□); 0.5 (■); 0.32 (△); 0.22 (▲); 0.16 (▽).

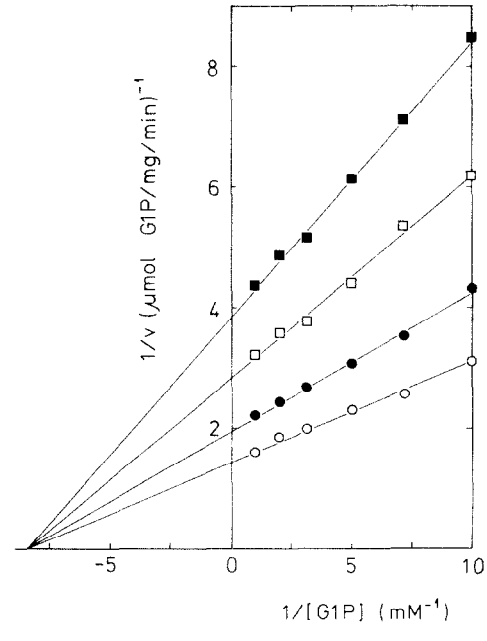

Fig. 3. Lineweaver–Burk plot of initial velocity of *Chlorella* phosphorylase against G1P at a series of fixed concentrations of amylopectin. The amylopectin concentrations were (mg/ml): 4.0 (○); 1.6 (●); 0.8 (□); 0.5 (■).

leaves, the latter being in the range 0.6–5 mM [16, 17, 19, 23].

Inhibition by ADP and ATP

In the direction of starch degradation in the presence of amylopectin of 2.0 mg/ml, ADP was shown to be a competitive inhibitor with respect to phosphate (Fig. 4). The replot of slope vs ADP concentration was a parabolic curve (Fig. 4), indicating that ADP is not a one-site, pure competitive inhibitor. In the direction of glucan synthesis by *Chlorella* phosphorylase in the presence of 2.0 mg/ml amylopectin, ADP was found to be a competitive inhibitor with respect to G1P (Fig. 5). The replot of slope vs ADP concentration also showed a parabola (Fig. 5). These

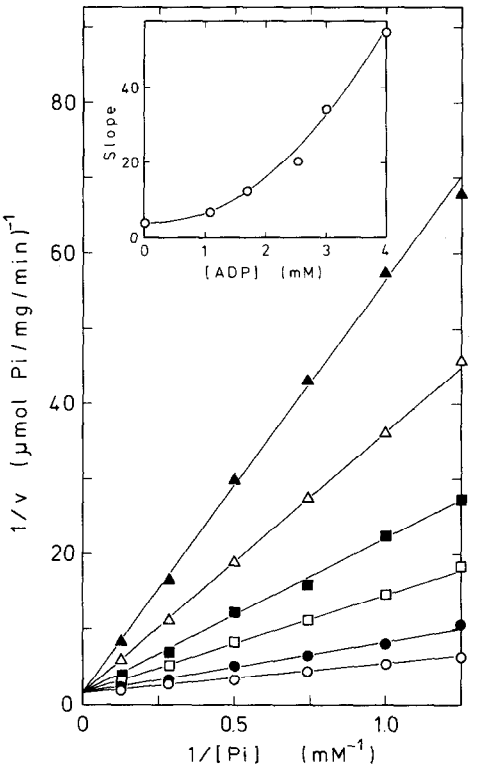

Fig. 4. Inhibition of *Chlorella* phosphorylase by ADP in the direction of phosphorolysis. Pi was the varied substrate with amylopectin held constant at 2.0 mg/ml. The concentrations of ADP (mM) were: 0 (○); 1.1 (●); 1.7 (□); 2.5 (■); 3.0 (△); 4.0 (▲).

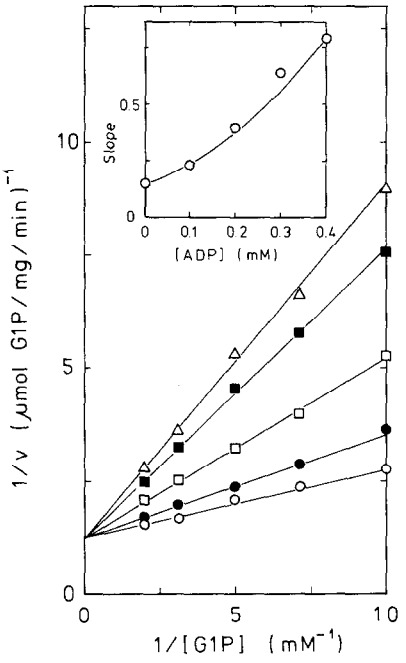

Fig. 5. Inhibition of *Chlorella* phosphorylase by ADP in the direction of polyglucan synthesis. G1P was the varied substrate with amylopectin held constant at 2.0 mg/ml. The concentrations of ADP (mM) were: 0 (○); 0.1 (●); 0.2 (□); 0.3 (■); 0.4 (△).

results suggest that ADP is an allosteric inhibitor of *Chlorella* phosphorylase in both directions. When the concentration of ADP was varied at a fixed concentration of Pi (2.4 mM, the K_m value for Pi) or at that of G1P (0.12 mM, the K_m value for G1P), the phosphorylase activity in either direction yielded a sigmoid curve (Fig. 6). The $I_{0.5}$ values for ADP, or ADP concentrations giving 50% inhibition, were 1.6 mM in the direction of glucan phosphorolysis and 0.23 mM in the direction of glucan synthesis, respectively. The Hill plots of the respective data indicated n values of 2.2 in the direction of starch degradation and of 1.5 in the direction of synthesis, respectively (Fig. 6).

For *Chlorella* phosphorylase in the directions of α-glucan degradation and synthesis, inhibitions by ATP were competitive toward Pi and G1P, respectively (data not shown). When the slopes of both Lineweaver-Burk plots were replotted against ATP concentrations, parabolic curves were obtained in both directions (data not shown). The plot of the phosphorylase activity against ATP concentration yielded sigmoid curves for both starch degradation and synthesis (Fig. 7). The $I_{0.5}$ value for ATP in the direction of phosphorolysis in the presence of 2.4 mM Pi was *ca* 2.9 mM, while the value in the direction of glucan synthesis in the presence of 0.12 mM G1P was estimated to be 1.4 mM. The Hill plots for *Chlorella*

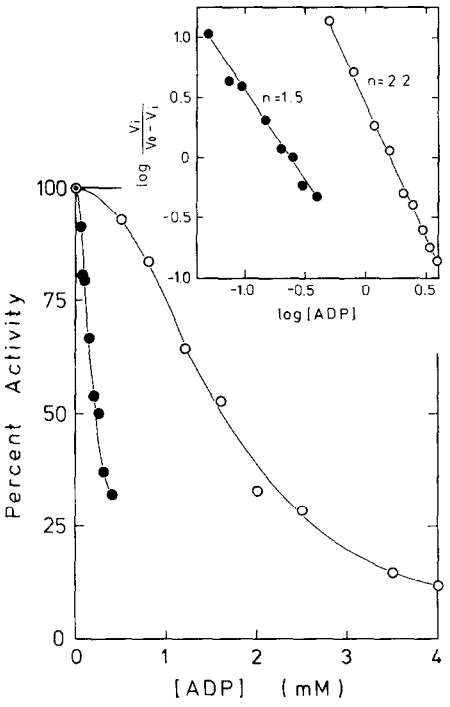

Fig. 6. Inhibition of *Chlorella* phosphorylase by ADP. The activity was assayed in the presence of various concentrations of ADP as described in Experimental, except that the concentration of Pi was kept at 2.4 mM in the direction of glucan phosphorolysis and that of G1P at 0.12 mM in the direction of the synthesis. Insert shows the Hill plot. V_0 and V_i show the activities in the absence and presence of ADP, respectively. (○) Phosphorylase activity in the direction of phosphorolysis; (●) synthesis.

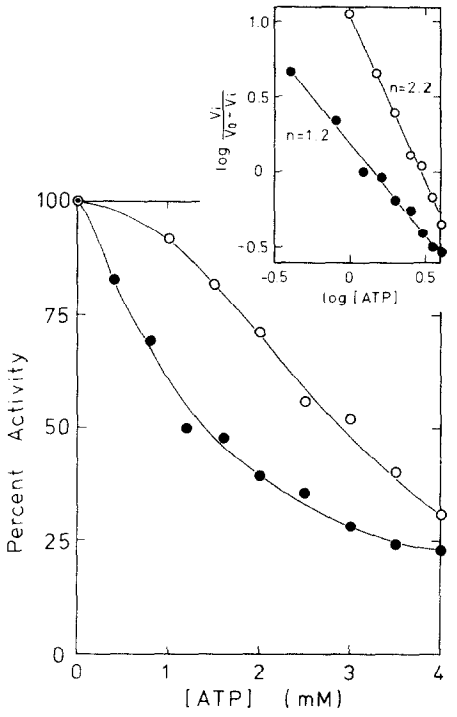

Fig. 7. Inhibition of *Chlorella* phosphorylase by ATP. The assay conditions were the same as described in Fig. 6, except that ADP was replaced by ATP. (○) Phosphorylase activity in the direction of phosphorolysis; (●) synthesis.

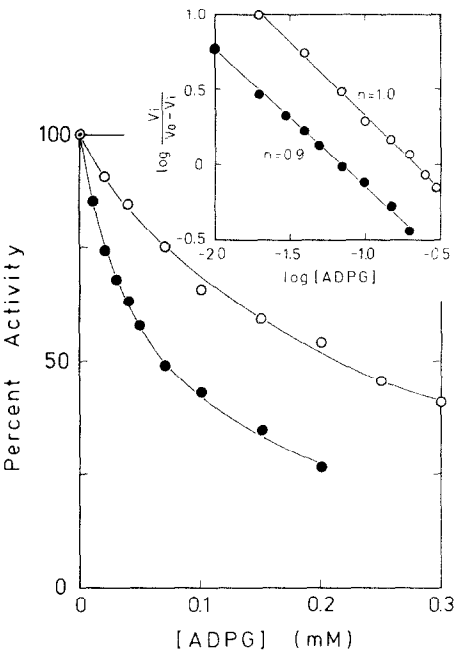

Fig. 8. Inhibition of *Chlorella* phosphorylase by ADPG. The assay conditions were the same as described in Fig. 6, except that ADP was replaced by ADPG. (○) Phosphorylase activity in the direction of phosphorolysis; (●) synthesis.

phosphorylase in the directions of starch phosphorolysis and synthesis gave n values of 2.2 and 1.2, respectively (Fig. 7).

Effects of other nucleotides

The following nucleotides did not significantly affect the activity of *Chlorella* phosphorylase in both directions: AMP, CMP, GMP, TMP, UMP, UDP and CTP (data not shown). GDP, GTP and UTP at 2 mM produced, respectively, 29, 14 and 25% inhibition of glucan phosphorolysis in the presence of 2.4 mM Pi, and inhibited, respectively, 67, 40 and 55% of glucan synthetic activities in the presence of 0.12 mM G1P.

Inhibition by ADPG and UDPG

ADPG was found to be a linear competitive inhibitor with respect to Pi in the direction of phosphorolysis (data not shown). The replots of slopes from the Lineweaver–Burk plot vs ADPG concentration gave a straight line and the K_i value for ADPG was estimated to be *ca* 0.11 mM. In the synthesis direction, ADPG was a linear competitive inhibitor with respect to G1P (data not shown). Similar replots of slopes against ADPG concentration yielded a straight line, with a K_i (ADPG) of *ca* 0.033 mM. Figure 8 shows that the curve of inhibition by ADPG of phosphorylase activity in the presence of 2.4 mM Pi or 0.12 mM G1P was a hyperbola. ADPG at *ca* 0.21 mM and 0.069 mM inhibited 50% of the activity in the directions of phosphorolysis and of synthesis, respectively. Each Hill plot gave a n value of 1.0 for phosphorolysis and of 0.9 for synthesis (Fig. 8). From these

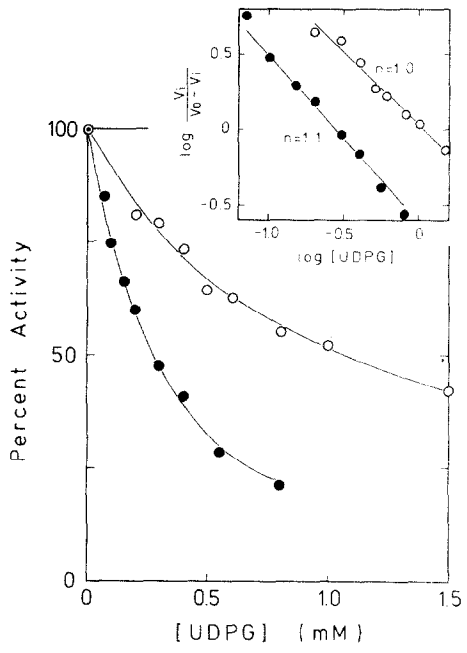

Fig. 9. Inhibition of *Chlorella* phosphorylase by UDPG. The assay conditions were the same as described in Fig. 6, except that ADP was replaced by UDPG. (○) Phosphorylase activity in the direction of phosphorolysis; (●) synthesis.

results, it is unlikely that inhibition by ADPG is co-operative.

UDPG was a linear competitive inhibitor of *Chlorella* phosphorylase in both directions with respect to Pi and G1P (data not shown). Similarly, the K_i value for UDPG was estimated to be 0.51 mM with respect to Pi and 0.15 mM with respect to G1P. As shown in Fig. 9, the $I_{0.5}$ values for UDPG in the direction of starch degradation in the presence of 2.4 mM Pi and in the direction of synthesis in the presence of 0.12 mM G1P, were calculated to be *ca* 1.1 mM and 0.27 mM, respectively. The n values of 1.0 in the direction of phosphorolysis and of 1.1 in the direction of synthesis were estimated from the respective Hill plots (Fig. 9).

In conclusion, the potent inhibition of *Chlorella* phosphorylase activity by ADP, ATP, ADPG or UDPG presents a striking contrast to previously reported results that those nucleotides and nucleotides sugars are little or less, if any, inhibitory to the activity of α-glucan phosphorylase from spinach leaves [15, 16, 19].

DISCUSSION

It has been generally accepted that starch is predominantly produced by the concerted action of ADP-glucose pyrophosphorylase and starch synthase, but not phosphorylase, during photosynthesis in chloroplasts of higher plant leaves and green algae [24]. The view that phosphorylase is not involved in starch biosynthesis in chloroplasts has been based on the following observations. Firstly, the K_m value for G1P for phosphorylase has been reported to be 10-fold higher than that for ADPG pyrophosphorylase, since the latter ranges from 0.04 mM in the presence of 3-phosphoglyceric acid to 0.1 mM in its absence [24]. Furthermore, the physiological concentrations of G1P under light conditions have been estimated to be *ca* 0.04 mM in *Chlorella pyrenoidosa* [25, 26] and 0.07–0.11 mM in spinach chloroplasts [3, 27].

However, the K_m value for G1P of *C. vulgaris* phosphorylase obtained in the present study, in contrast to those of other plant enzyme, appears to be comparable to the K_m value for G1P of ADPG pyrophosphorylase and to the physiological concentration of G1P in green plants. In addition, the optimum activity of phosphorylase has been reported to be *ca* three-fold in excess of that of ADPG pyrophosphorylase and 10–20-fold in excess of that of starch synthase in certain plant tissues [28]. Therefore, one can not exclude the possibility that, at least in certain physiological conditions, phosphorylase is functional in starch biosynthesis as well as in its degradation in *C. vulgaris*.

There have been a number of studies on the kinetic properties of α-glucan phosphorylase extracted from a variety of higher plant tissues. It is known that phosphorylase from many of the plant sources investigated shows no sign of allosteric or regulatory properties.

No significant inhibition was found with ATP, ADP or AMP in the activity of phosphorylase from maize seeds [29] and mature pea seeds [22]. Burr and Nelson [30] reported that ATP did not affect the activity of phosphorylase from developing maize seeds in the direction of starch degradation while this compound inhibited the activity in the direction of the synthesis, K_i value for ATP being 5 mM. The inhibition of three isozymes of phosphorylase from banana fruit was tested in the direction of glucan synthesis [31]. Although 5 mM ATP inhibited one isozyme by 90%, the activities of the other forms remained unaltered by the addition of ATP. In contrast, there have been previous reports of no inhibition by 1–5 mM ATP and ADP of two types of phosphorylase, the chloroplast and cytoplasmic enzymes, from spinach leaves [15, 16, 19] and from broad bean cotyledons [15] either in the direction of starch synthesis or in its degradation.

The present data clearly show that both ADP and ATP are potent inhibitors of phosphorylase from *C. vulgaris* within physiological concentrations (Figs. 4–7). Both nucleotides seem to be allosteric inhibitors of the enzyme since the inhibition curves do not follow Michaelis–Menten kinetics and are sigmoid-shaped (Figs. 6 and 7). The $I_{0.5}$ values for ATP of 2.9 mM in the direction of glucan degradation and of 1.4 mM in the direction of synthesis and the value for ADP of 1.6 mM in the direction of phosphorolysis are similar, or a little lower, compared with those values shown in other plant tissues where inhibition occurs. However, it should be mentioned that the $I_{0.5}$ value for ADP of 0.23 mM in the direction of starch synthesis by *C. vulgaris* phosphorylase appears to be much lower than the previously reported values.

In many nonchlorophyllous plant tissues, e.g. potato tubers [32], developing maize seeds [30], mature and germinated pea seeds [22] and banana fruit [31], K_i (UDPG) values for α-glucan phosphorylase have been in the range 2–3 mM. Starch phosphorylase from potato tubers [32], banana fruit [31] and mature pea seeds [22] has been reported to be unaffected by ADPG, whereas 50% of the activity of the enzyme from corn seeds [30] and germinated pea seeds [22] has been shown to be inhibited by 1–2 mM ADPG. On the other hand, in higher plant leaves both ADPG and UDPG have been shown to be poor inhibitors of the chloroplast phosphorylase as well as of the cytoplasmic enzyme [15, 16, 19].

However, the present data (Figs. 8 and 9) indicate that both ADPG and UDPG are linear competitive inhibitors of *C. vulgaris* phosphorylase. Interestingly, the estimated K_i values for UDPG of 0.51 mM in the direction of phosphorolysis and of 0.15 mM in the direction of synthesis are considerably lower than those reported for the nonchlorophyllous tissues described above. In addition, it is noteworthy that ADPG is a strong inhibitor of the *C. vulgaris* enzyme. The K_i values for ADPG of 0.11 mM in the direction of phosphorolysis and of 0.033 mM in the direction of synthesis seem to be much lower than those previously reported in other plant tissues.

It is likely that the concentration of ADPG is almost 0.4 mM in *C. pyrenoidosa* [26]. In spinach chloroplasts the ADPG concentration was found to be up to 0.08 mM [3]. The K_m (ADPG) values for *C. pyrenoidosa* ADPG pyrophosphorylase were found to be 1.8 mM in the presence of 1 mM 3-phosphoglyceric acid and 2.8 mM in its absence [33] and the value for *C. pyrenoidosa* starch synthase was *ca* 0.27 mM [34]. Based on these observations, the above described K_i values for ADPG for *C. vulgaris* phosphorylase seem to be well within the physiological concentrations of ADPG.

EXPERIMENTAL

Algal material. C. vulgaris 11 h cells were grown photoautotrophically in an inorganic culture medium at *ca* 23° with bubbling with 3% CO_2 in air as described previously [35].

Harvested cells were washed once with 100 mM Tris–HCl buffer, pH 7.4, contained 2 mM DTT, 5 mM $MgCl_2$ and 2 mM EDTA, and suspended in 30 ml of the same medium at a cell density of 0.12 ml packed cell vol./ml.

Preparation of Chlorella *phosphorylase.* The following procedures were carried out at 4°, unless otherwise described. The *Chlorella* cells were disrupted twice in a French pressure cell at 500 kg/cm^2. The resulting homogenate was centrifuged at 50 000 *g* for 90 min. To 24 ml of the supernatant fraction was slowly added 10 ml of a satd $(NH_4)_2SO_4$ soln. The ppt was discarded and 44 ml more $(NH_4)_2SO_4$ soln was added. The resulting suspension was centrifuged at 12 000 *g* for 30 min. The ppt thus obtained was dissolved in 50 mM imidazole–HCl buffer, pH 7.0, containing 0.1 mM DTT, in a final vol. of 2.8 ml and was dialysed overnight against 500 ml of the same buffer.

The dialysed fraction (2.5 ml) was applied onto a 2.2 × 30 cm DEAE-cellulose (Whatman DE-52) column, which had been equilibrated with 50 mM imidazole–HCl buffer, pH 7.0, containing 0.1 mM DTT. The column was washed with 10 ml of the same buffer, and then eluted with a linear gradient of from 0 to 0.5 M NaCl in this buffer (300 ml in each reservoir). Fractions of 180 drops (*ca* 6.2 ml) were collected and assayed for enzyme activity and protein content. The fractions constituting the peak of phosphorylase activity were pooled and stored on ice before use. The enzyme activity was stable for at least 3 months at 0°. However, the activity was completely lost when the sample was stored in a freezer (−20°) and then thawed.

Assay of phosphorylase. The activity in the direction of phosphorolysis was assayed by the rate of glucose 1-[^{32}P] phosphate formation from $H_3{}^{32}PO_4$ in the presence of amylopectin by the method of Avron [36]. The reaction mixture usually contained 50 mM glycylglycine buffer, pH 7.1, 1.0 mg potato amylopectin, 8.0 mM $K_2H^{32}PO_4$ and the partially purified enzyme (1.7 μg protein), in a final vol. of 0.5 ml. The reaction was started by adding the enzyme fraction. The reaction was run at 30° for 15 min and terminated by adding 0.3 ml 20% trichloroacetic acid.

The activity in the direction of α-glucan synthesis was measured by the incorporation of ^{14}C from [U-^{14}C]glucose 1-phosphate into starch. The reaction soln usually consisted of 50 mM MES buffer, pH 6.2, 1.0 mg potato amylopectin, 1.0 mM [U-^{14}C]glucose 1-phosphate and the partially purified enzyme (0.9 μg protein), in a final vol. of 0.5 ml. The reaction was started by adding the enzyme fraction, run for 10 min at 30° and stopped by adding 2.0 ml MeOH. The sample was centrifuged at 3000 rpm for 20 min and the resulting ppt washed with 2.0 ml 80% MeOH. The 80% MeOH-insoluble fraction was suspended in 0.5 ml 20% MeOH, homogenized and then taken into a scintillation glass vial. The amount of radioactivity was measured in 4 ml scintillating cocktail ACS II (Amersham) in a liquid scintillation counter.

When [^{14}C]G1P at concns in the range used in the phosphorolytic assay was incubated with the enzyme preparation (2 μg protein) in the absence of amylopectin for 15 min at 30° in a vol. of 0.5 ml, neither [^{14}C]glucose nor glucose 6-phosphate was found to be produced. This indicates the absence of phosphatase and phosphoglucomutase in the preparation. When [^{14}C]amylopectin, synthesized from [^{14}C]G1P and amylopectin in the presence of the partially purified phosphorylase, was incubated with the phosphorylase preparation in the absence of Pi, no radioactivity was released into the MeOH-soluble fraction. This indicates that the preparation was free of amylases that would degrade any newly synthesized starch.

Acknowledgements—We are grateful to Dr. S. Miyachi for his encouragement and to Dr. Y. Satoh for helpful discussion.

REFERENCES

1. Levi, C. and Gibbs, M. (1976) *Plant Physiol.* **57**, 933.
2. Steup, M., Peavey, D. G. and Gibbs, M. (1976) *Biochem. Biophys. Res. Commun.* **72**, 1554.
3. Heldt, H. W., Chon, C. J., Maronde, D., Herold, A., Stankovic, Z. S., Walker, D. A., Kraminer, A., Kirk, M. R. and Heber, U. (1977) *Plant Physiol.* **59**, 1146.
4. Peavey, D. G., Steup, M. and Gibbs, M. (1977) *Plant Physiol.* **60**, 305.
5. Levi, C. and Preiss, J. (1978) *Plant Physiol.* **61**, 218.
6. Stitt, M., Bulpin, P. V. and apRees, T. (1978) *Biochim. Biophys. Acta* **544**, 200.
7. Stitt, M. and apRees, T. (1980) *Biochim. Biophys. Acta* **627**, 131.
8. Stitt, M. and Heldt, H. W. (1981) *Plant Physiol.* **68**, 755.
9. Nakamura, Y. and Miyachi, S. (1977) in *Biological Solar Energy Conversion* (Mitsui, A., Miyachi, S., San Pietro, A. and Tamura, S., eds.) p. 203. Academic Press, New York.
10. Nakamura, Y. and Miyachi, S. (1982) *Plant Cell Physiol.* **23**, 333.
11. Okita, T. W., Greenberg, E., Kuhn, D. N. and Preiss, J. (1979) *Plant Physiol.* **64**, 187.
12. Steup, M. and Latzko, E. (1979) *Planta* **145**, 69.
13. Steup, M., Schächtele, C. and Latzko, E. (1980) *Z. Pflanzenphysiol.* **96**, 365.
14. Kombrink, E. and Wöber, G. (1980) *Planta* **149**, 130.
15. de Fekete, M. A. R. (1968) *Planta* **79**, 208.
16. Preiss, J., Okita, T. W. and Greenberg, E. (1980) *Plant Physiol.* **66**, 864.
17. Kumar, A. and Sanwal, G. G. (1981) *Indian J. Biochem. Biophys.* **18**, 114.
18. Steup, M. and Schächtele, C. (1981) *Planta* **153**, 351.
19. Shimomura, S., Nagai, M. and Fukui, T. (1982) *J. Biochem.* **91**, 703.
20. Tsai, C. Y. and Nelson, O. E. (1968) *Plant Physiol.* **43**, 103.
21. Richardson, R. H. and Matheson, N. K. (1977) *Phytochemistry* **16**, 1875.
22. Graves, D. J. and Wang, J. H. (1972) in *The Enzymes* (Boyer, P. D., ed.), 3rd edn, p. 435. Academic Press, New York.
23. Matheson, N. K. and Richardson, R. H. (1978) *Phytochemistry* **17**, 195.
24. Preiss, J. and Levi, C. (1980) in *The Biochemistry of Plants* (Preiss, J., ed.) Vol. 3, p. 371. Academic Press, New York.
25. Bassham, J. A. and Krause, G. H. (1967) *Biochim. Biophys. Acta* **189**, 207.
26. Kanazawa, T., Kanazawa, K., Kirk, M. R. and Bassham, J. A. (1972) *Biochim. Biophys. Acta* **256**, 656.
27. McLilley, R., Chon, C. J., Moobach, A. and Heldt, H. W. (1977) *Biochim. Biophys. Acta* **460**, 259.
28. Ozbun, J. L., Hawker, J. S., Greenberg, E., Lammel, L., Preiss, J. and Lee, E. Y. C. (1973) *Plant Physiol.* **51**, 1.
29. Lee, E. Y. C. and Braun, J. J. (1973) *Arch. Biochem. Biophys.* **156**, 276.
30. Burr, B. and Nelson, O. E. (1975) *Eur. J. Biochem.* **56**, 539.
31. Singh, S. and Sanwal, G. G. (1976) *Phytochemistry* **15**, 1447.
32. de Fekete, M. A. R. and Cardini, C. E. (1964) *Arch. Biochem. Biophys.* **104**, 173.
33. Sanwal, G. G. and Preiss, J. (1967) *Arch. Biochem. Biophys.* **119**, 454.
34. Preiss, J. and Greenberg, E. (1967) *Arch. Biochem. Biophys.* **118**, 702.
35. Nakamura, Y. and Miyachi, S. (1980) *Plant Cell Physiol.* **21**, 765.
36. Avron, M. (1960) *Biochim. Biophys. Acta* **40**, 257.