ADENINE NUCLEOTIDE LEVELS IN CELLS OF THE MARINE ALGA, *GRIFFITHSIA**

By R. McC. Lilley† and A. B. Hope†

The ionic relations of giant cells of the marine red alga *Griffithsia* are determined, in part, by an active inward transport of Cl^- ions that is stimulated by light (Findlay, Hope, and Williams 1969, 1970). The light-dependent component of this active Cl^- influx is affected by metabolic inhibitors in a manner suggesting that the energy supply for it is derived from non-cyclic photophosphorylation (Lilley and Hope 1971).

This conclusion was partly based on an assumption that the availability of ATP in the cell is greater in the light, due to photosynthesis, than in the dark. However, the steady-state levels of ATP in cells of a range of algae (Holm-Hansen 1970), and in *Chara foetida* (Penth and Weigl 1971), have recently been shown to be similar in the light and dark.

It was therefore decided to measure the concentrations of ATP, ADP, and AMP in cells of *Griffithsia monile*, and to study the effects of darkness and of metabolic inhibitors on these concentrations and on the adenine nucleotide energy charge (Atkinson 1969).

Methods

The collection and storage of *G. monile* and the composition of the artificial seawater has been previously described (Lilley and Hope 1971). All experimental solutions used from the commencement of pretreatment to the extraction of nucleotides contained $1{\cdot}6 \times 10^{-4}$M chloramphenicol (Sigma) as a precaution against bacterial contamination of the cells (Leaver and Edelman 1965), although a preliminary assay showed that this made no difference to their ATP content.

Short strands of 5–10 cells were pretreated in artificial seawater for 12 hr before the experiment. Batches of approximately 20 cells were measured for determination of surface area (Findlay, Hope, and Williams 1969), then treated for 2 hr under the conditions shown at 22°C. The light source was a bank of four 40-W white fluorescent tubes. The cells were then rapidly transferred into 1·0 ml 0·4M perchloric acid, 1 mM EDTA at 2°C in a running Ultraturrax homogenizer which was stopped after 3 sec, although disruption of the cells was apparently instantaneous. The homogenates were centrifuged at 20,000 *g* for 20 min, the supernatant neutralized with 1·0M KOH, 0·1M Tris (Sigma), and then centrifuged at 20,000 *g* for 10 min, all at 2°C. The supernatant was assayed immediately.

The ATP content of samples was measured by the firefly luciferase method. Purified luciferin and luciferase were prepared from desiccated firefly tails (Calbiochem) by Sephadex gel filtration, and used in the assay by the method of Nielsen and Rasmussen (1968). The light

* Manuscript received June 10, 1971.

† School of Biological Sciences, Flinders University of South Australia, Bedford Park, S.A. 5042.

emission was measured in a Nuclear-Chicago Mark I scintillation spectrometer by a procedure similar to that of Stanley and Williams (1969). The ATP+ADP content of extracts was determined by measuring ATP after incubating a 0·2-ml sample with 25 nmoles phosphoenolpyruvate (Calbiochem) and 8 μg pyruvate kinase (Boehringer) for 15 min at 30°C. The ATP+ADP+AMP content was determined on an identical reaction mixture with the addition of 25 μg adenylate kinase (Boehringer). All assays were done with internal standards to compensate for quenching of luminescence by the sample (usually 20–30%), and to check that enzymic conversion to ATP was complete.

TABLE 1

ADENINE NUCLEOTIDES IN CELLS OF *G. MONILE*

Adenine nucleotide concentrations expressed as pmoles cm^{-2} cell surface area

Conditions	ATP	ADP	AMP	Total Adenylate	Energy Charge*
Experiment 1					
Light	374	55	167	596	0·67
Dark	434	103	153	690	0·70
Light, 3 μM DCMU	334	114	133	581	0·67
Light (repeat)	429	62	212	703	0·65
Experiment 2					
Light	451	32	182	665	0·70
Dark	392	71	147	610	0·70
Light, 5 μM CCCP	474	118	4	596	0·89
Dark, 5 μM CCCP	385	96	190	671	0·65
Experiment 3					
Light	334	113	115	562	0·69
Dark	354	38	129	521	0·72
Light, 10 μM CCCP	318	42	73	433	0·78
Dark, 10 μM CCCP	187	221	175	583	0·51
Experiment 4					
Light	266	16	92	374	0·73
Dark	172	50	64	286	0·69
Light, 10 mM iodoacetamide	94	47	148	289	0·41
Dark, 10 mM iodoacetamide	45	57	204	306	0·24
Pooled means (±S.E.M.)					
Light (5 determinations)	371±33	56±16	154±22	581±57	0·69±0·02
Dark (4 determinations)	338±57	66±14	123±20	527±87	0·70±0·01

* Energy charge $= \frac{[ATP]+\frac{1}{2}[ADP]}{[ATP]+[ADP]+[AMP]}$ (Atkinson 1969).

Results and Discussion

Table 1 shows the results of four experiments in which the effects of darkness, and of metabolic inhibitors on the adenine nucleotide levels were measured. The most appropriate method of routinely expressing these concentrations appeared to be as picomoles of nucleotide per 1 cm^2 cell surface area, as the cytoplasm in *G. monile* forms a thin, even layer (approx. 5 μm thick) around the vacuole. Within each experiment, there appears to be no consistent difference in the concentrations of any of the adenine nucleotides, or in the energy charge, between cells from the light and dark. The pooled means of these values also show no significant difference between light and dark. A typical value for the chlorophyll *a* content of cells of *G. monile*

is 1·4 μg chlorophyll *a* per 1 cm^2 (Lilley, unpublished data). From this, the average ATP concentration in illuminated *G. monile* cells on a chlorophyll basis is 265 pmoles ATP per microgram chlorophyll *a*.

The results of experiment 1 show that 3 μM (3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) has no apparent effect on ATP concentration or the energy charge. 3 μM DCMU inhibits apparent photosynthetic oxygen evolution and carbon fixation to less than 5% of the control rates in *G. monile* (Lilley and Hope 1971). These results show that neither the inhibition of non-cyclic electron flow by DCMU, nor the complete inhibition of photosynthesis by darkness, cause any change in the total cell ATP concentration or energy charge. This suggests that ATP and the adenylate energy charge are closely regulated in these cells, rather than being the result of a fortuitous balance between ATP produced in photophosphorylation and that consumed by carbon fixation and other light-dependent ATP-consuming processes. This is particularly evident when the rate of photophosphorylation is considered. Cells of *G. monile* evolve, typically, 60 pmoles O_2 cm^{-2} sec^{-1} in the light (Lilley and Hope 1971). Assuming that 3 ADP are phosphorylated per O_2 evolved, then the rate of photophosphorylation is 180 pmoles ATP cm^{-2} sec^{-1}, or approximately half of the total cell ATP per second. To maintain a constant level both in light and dark with such rapid turnover of ATP in the light would seem to require an efficient regulatory mechanism. It has been proposed by Atkinson (1969) that such control is provided by major metabolic pathways in the cell that are amphibolic in nature, such as glycolysis. The present evidence for interaction of adenylates with glycolysis in plant cells has been summarized by Heber and Santarius (1970).

Experiments 2 and 3 show that carbonyl cyanide m-chlorophenylhydrazone (CCCP) causes an increase in ATP level and in the energy charge in the light, the effect being greater with 5 μM than with 10 μM CCCP. 5 μM CCCP partially uncouples photosynthesis in *G. monile* judged by its effects on oxygen evolution and carbon fixation, and probably also uncouples respiration (Lilley and Hope 1971). Under these conditions, one reason for an increase in the adenylate energy charge could be an increase, by some unknown mechanism, in the rate of substrate-level phosphorylation in the cell. This effect must be light-dependent as CCCP lowers the ATP level and energy charge in the dark.

Experiment 4 shows that 10 mM iodoacetamide lowers the ATP level and energy charge in the light and, to a greater extent, in the dark. Iodoacetamide probably affects most metabolic processes in the cell, and may have inhibited metabolic pathways involved in regulation of ATP levels. Under these conditions, the ATP level and energy charge have become light-dependent.

Conclusions

Although the steady-state level of ATP and the adenylate energy charge in illuminated cells of *G. monile* are the same as in darkness, transient changes on initial illumination of the type observed in other plant cells (Holm-Hansen 1970; Heber and Santarius 1970) probably occur. No attempt has been made here to measure separately the adenylate levels in chloroplasts and cytoplasm. It has, however, been shown that in a number of plant tissues, adenylates are readily transferred between chloroplasts and cytoplasm (Heber and Santarius 1970).

The results show that the light-dependent, active Cl^- influx in *G. monile*, if dependent on ATP at all, is not controlled by ATP availability in the cell. It seems likely that the rate of active Cl^- influx, and the supply of energy to drive it, may be controlled by separate mechanisms.

Acknowledgments

We wish to thank Professor A. W. Murray for his helpful advice. R.M.L. acknowledges the receipt of a Commonwealth Postgraduate Award. The project was supported by the Australian Research Grants Committee.

References

ATKINSON, D. E. (1969).—*A. Rev. Microbiol.* **23**, 47.
FINDLAY, G. P., HOPE, A. B., and WILLIAMS, E. J. (1969).—*Aust. J. biol. Sci.* **22**, 1163.
FINDLAY, G. P., HOPE, A. B., and WILLIAMS, E. J. (1970).—*Aust. J. biol. Sci.* **23**, 323.
HEBER, U., and SANTARIUS, K. A. (1970).—*Z. Naturf.* **25b**, 718.
HOLM-HANSEN, O. (1970).—*Pl. Cell Physiol.* **11**, 689.
LEAVER, C. J., and EDELMAN, J. (1965).—*Nature, Lond.* **207**, 1000.
LILLEY, R. McC., and HOPE, A. B. (1971).—*Biochim. Biophys. Acta* **226**, 161.
NIELSEN, R., and RASMUSSEN, H. (1968).—*Acta chem. scand.* **22**, 1757.
PENTH, B., and WEIGL, J. (1971).—*Planta* **96**, 212.
STANLEY, P. E., and WILLIAMS, S. G. (1969).—*Analyt. Biochem.* **29**, 381.