Phytochemistry, 1979, Vol. 18, pp. 2015–2017. © Pergamon Press Ltd. Printed in England. 0031-9422/79/1201-2015 $02.00/0

SHORT REPORTS

CYCLIC-AMP CONTROL OF SOME OXIDO-REDUCTASES DURING PINE POLLEN GERMINATION AND TUBE GROWTH

ASHOK K. DHAWAN* and CHANDER P. MALIK
Department of Botany, Punjab Agricultural University, Ludhiana, India

(*Revised received* 30 *April* 1979)

Key Word Index—*Pinus roxburghii*; Pinaceae; pollen; oxido-reductases; cyclic-AMP.

Abstract—Cyclic-AMP markedly increased the activities of peroxidase, malate dehydrogenase and succinate dehydrogenase but not glucose-6-phosphate dehydrogenase. Using inhibitors of protein and RNA synthesis, it was found that a part of enzyme activity increase caused by cyclic-AMP required fresh protein synthesis. The question of specificity of enzyme induction by cyclic-AMP has been examined.

INTRODUCTION

Earlier work from this laboratory has shown that cyclic-AMP enhances pollen germination, increases tube length, and also has a synergistic effect in combination with GA [1, 2]. This is interesting since hormones are known to affect pollen tube growth [3, 4] and cyclic-AMP might, therefore, be acting as a secondary messenger in their action.

Here we report a possible manner in which cyclic-AMP might be regulating the pollen tube growth. Since a large number of oxido-reductases have been shown to be associated with the process of pollen tube growth [4–6], an investigation was planned to study the effect of cyclic-AMP on these enzymes. This has yielded information, which appears to be important in explaining the control of enzyme activities in pollen, besides giving leads to the manner in which cyclic-AMP might be regulating the pollen tube growth.

RESULTS

Pollen tube length was markedly enhanced by cyclic-AMP, while 5′-AMP was ineffective (Table 1).

Fig. 1 shows the effect of cyclic-AMP on the activities of 4 oxido-reductases. The peroxidase and malate dehydrogenase activities, which decrease with pollen germination in the control, markedly increase at 20 hr in the cyclic-AMP treated pollen. While 5′-AMP had only a slight effect on the peroxidase activity, it increased the malate dehydrogenase activity. The trends of activity of succinate dehydrogenase were similar to those of peroxidase, except that its activity increased with pollen germination and the cyclic-AMP effect was most pronounced at 40 hr. Glucose-6-phosphate dehydrogenase was not affected by cyclic-AMP or 5′-AMP.

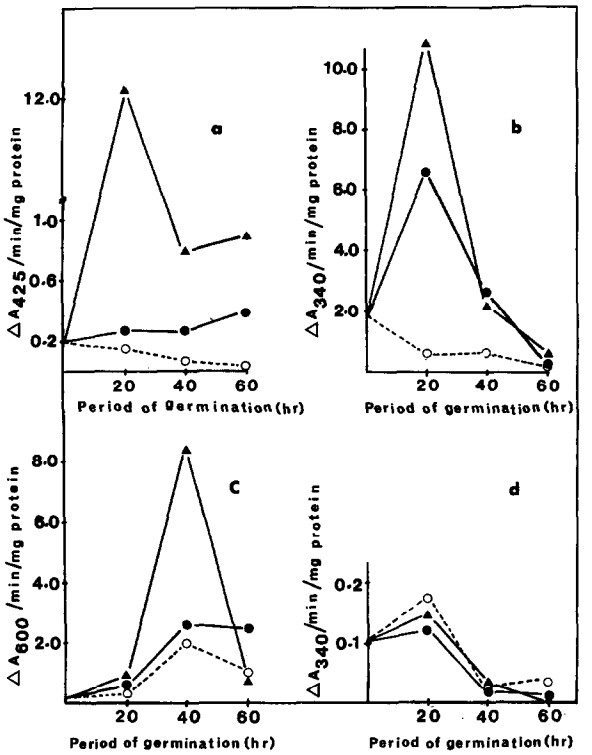

Fig. 1. Peroxidase (a), malate dehydrogenase (b), succinate dehydrogenase (c) and glucose-6-phosphate dehydrogenase (d) activities at different stages of *Pinus roxburghii* pollen cultured in 1 mg/l. of each of 5′-AMP (●——●) and cyclic-AMP (▲——▲). 50 mM sucrose was used as the basal media (○——○).

Table 2 shows the effect of 10 mg/l. cycloheximide and rifamycin on the malate dehydrogenase activity. Application of 10 mg/l. cycloheximide at zero hr of incubation

* Present address and to whom correspondance should be addressed: Botany Department, The Australian National University, P.O. Box 4, Canberra, ACT 2600, Australia.

Table 1. Effect of cyclic-AMP and 5'-AMP (1 mg/l. each) on the *Pinus roxburghii* pollen tube growth (μm) 60 hr after incubation

Treatment	Pollen tube length*
Control	75.8 ± 4.0
Cyclic-AMP	207.0 ± 9.3
5'-AMP	84.7 ± 3.2

Sucrose (50 mM) was used as basal media. Pollen tube emergence starts 20 hr after incubation of the pollen.
* Means ± S.E.

effectively decreased the enzyme activity. Rifamycin treatment affected the enzyme activity at zero hr, but not at 10 hr after incubation. Cyclic-AMP treatment, which markedly increased the malate dehydrogenase activity, was less effective in the presence of 10 mg/l. rifamycin or cycloheximide. It should be noted, however, that although the enzyme activity in the presence of cycloheximide + cyclic-AMP or rifamycin + cyclic-AMP remained more than the control (sucrose media alone), the per cent decrease in enzyme activity caused by these inhibitors was more in the presence of cyclic-AMP than without it (cf. enzyme activities as percent of the respective controls).

DISCUSSION

The fact that cyclic-AMP caused increase in pollen tube length (Table 1) is interesting, since plant hormones like IAA, GA and ethylene are known to promote pollen tube length in this system [7]. Although cyclic-AMP mimics the effect of plant hormones in a similar manner in many other physiological responses [8, 9], the only other known examples of cyclic-AMP effect in pollen germination are of *Arachis* and *Tradescantia* pollen [1, 2].

Cyclic-AMP effectively enhanced the activities of peroxidase, malate dehydrogenase and succinate dehydrogenase, but not glucose-6-phosphate dehydrogenase (Fig. 1). While peroxidase and many other hydrolytic enzymes are known to be affected by cyclic-AMP, its effect on malate dehydrogenase and succinate dehydrogenase is being reported for the first time. This, therefore, raises a possibility that cyclic-AMP may be exerting its influence through the TCA-cycle to cause various physiological responses. It may be of interest to add here that cyclic-AMP has also been recently shown to affect the rate of CO_2 fixation in plant cells [10].

It is interesting to note that the peroxidase and malate dehydrogenase activities which decrease as the pollen germination proceeds, markedly increase at 20 hr of pollen germination in the presence of cyclic-AMP. This is an important indication of specificity in the control of these enzymes, i.e. cyclic-AMP-caused enzyme activity increase is not merely a consequence of growth triggered by the induction of pollen germination, but a specific event in the cyclic-AMP mediated pollen development. Glucose-6-phosphate dehydrogenase activity which increases with pollen germination, but was not affected by cyclic-AMP, also supports a specificity in the cyclic nucleotide action.

The malate dehydrogenase activity increase seems to require a fresh protein synthesis, since cycloheximide treatment of the pollen at zero hr decreases the enzyme activity (Table 2). Interestingly, the malate dehydrogenase activity in the presence of cyclic-AMP + rifamycin remained higher than the control pollen (sucrose-germinated). Presumably presynthesized RNAs are involved in this type of response.

Based on the above observations, it appears that the cyclic-AMP-caused enhancement of the pollen germination may be in part due to its effect on the oxido-reductases. Further, a specific increase in the activities of peroxidase and malate dehydrogenase suggests that cyclic-AMP might, perhaps, be one of the factors controlling the activities of these enzymes in plant systems.

EXPERIMENTAL

Pinus roxburghii Sarg pollen collected from uniformly-sized, freshly dehisced cones was stored at −5° in a desiccator. Pollen (100 mg) was germinated in 10 ml 50 mM sucrose in a conical flask with a cotton plug and placed in a growth chamber with four 40 W fluorescent tubes at a temp. of 28 ± 2°. Pollen tube length was recorded under a microscope fitted with an ocular micrometer.

Enzyme assays. After requisite treatment, the basal media were removed by filtration. The pollen was washed thoroughly with H_2O and extracted in 50 mM Pi buffer, pH 7 containing 2.5 mM cysteine hydrochloride and 0.25 mM EDTA. The supernatant, after centrifugation at 10000 *g*, was used for the assay of enzyme activities.

The peroxidase (EC 1.11.1.7) activity was assayed following the method of ref. [11], malate dehydrogenase (EC 1.1.1.37) by the method of ref. [12], succinate dehydrogenase (EC 1.3.99.1) by the method of ref. [13], glucose-6-phosphate dehydrogenase

Table 2. Effect of cycloheximide and rifamycin (10 mg/l. each) on the malate dehydrogenase activity* (ΔA_{340}/min/mg protein)

		Zero hr block		10 hr block	
	Control	Cycloheximide	Rifamycin	Cycloheximide	Rifamycin
Control	0.95 ± 0.10 (100)	0.38 ± 0.09 (39.8)	0.69 ± 0.12 (72.2)	0.82 ± 0.11 (86.4)	0.92 ± 0.10 (96.5)
Cyclic-AMP	9.78 ± 0.88 (100)	2.85 ± 0.32 (29.1)	2.95 ± 0.17 (30.2)	4.25 ± 0.50 (43.5)	6.85 ± 0.57 (70.0)

Pollen was cultured in 50 mM sucrose as the basal media with or without 1 mg/l. cyclic-AMP. Cycloheximide or rifamycin was added at 0 and 10 hr after incubation of the pollen in the media and the enzyme activities measured at 20 hr in each case. Figures in parentheses show the enzyme activity as per cent of its respective control.
* Mean ± S.E.

(EC 1.1.1.49) by the method of ref. [14]. Total proteins were estimated by the method of ref. [15].

Acknowledgements—This work has been financed by the Indian Council of Agricultural Research, New Delhi, and forms a part of the Ph.D. Thesis submitted to the Punjab Agricultural University, Ludhiana, by A. K. D.

REFERENCES

1. Malik, C. P., Chhabra, N. and Vermani, S. (1976) *Biochem. Physiol. Pflanz.* **168**, 311.
2. Malik, C. P. and Chhabra, N. (1976) *Proc. Indian Acad. Sci.* **84B**, 101.
3. Johri, B. M. and Vasil, I. K. (1961) *Bot. Rev.* **27**, 325.
4. Stanley, R. G. and Linskens, H. F. (1974) *Pollen Biology, Biochemistry, and Management*. Springer, Berlin.
5. Malik, C. P. (1977) in *Advances in Pollen and Spore Research* (Nair, P. K. K., ed.) Vol. II, p. 54. Today and Tomorrow, New Delhi.
6. Brewbaker, J. L. (1971) in *Pollen: Development and Physiology* (Heslop-Harrison, J., ed.) p. 156. Butterworth, London.
7. Dhawan, A. K. (1977) Ph.D. Thesis, Punjab Agricultural University, Ludhiana.
8. Amerhein, N. (1977) *Annu. Rev. Plant Physiol.* **28**, 123.
9. Sachar, R. C., Taneja, S. R. and Sachar, K. (1975) *J. Sci. Ind. Res.* **34**, 54.
10. Wiedmair, J. and Kull, U. (1978) *Biochem. Physiol. Pflanz.* **172**, 421.
11. Shannon, L. M., Kay, E. and Jew, J. K. (1966) *J. Biol. Chem.* **241**, 2126.
12. Antonn, M. D. and Roberts, M. F. (1975) *Phytochemistry* **14**, 909.
13. Haitt, A. J. (1961) *Plant Physiol.* **36**, 552.
14. Komamine, A. and Shimizu, T. (1975) *Physiol. Plant.* **33**, 47.
15. Lowry, D. H., Rosebrough, N. J., Farr, A. L. and Randall, J. L. (1951) *J. Biol. Chem.* **193**, 265.

Phytochemistry, 1979, Vol. 18, pp. 2017–2019. Pergamon Press Ltd. Printed in England. 0031-9422/79/1201-2017 $02.00/0

BEHAVIOUR OF SOME MITOCHONDRIAL ENZYMES IN RIPENING TOMATO FRUIT

SUSHIL K. KALRA* and JAMES L. BROOKS†

* Central Mango Research Station, Lucknow, India-226006 and
† Division of Plant Sciences, West Virginia University, Morgantown, WV 26506, U.S.A.

(*Revised received* 18 *April* 1979)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato fruit; ripening process; mitochondrial enzymes.

Abstract—The activities of four mitochondrial enzymes were studied in four stages of ripening tomato fruit. The highest enzyme activity was recorded for malate dehydrogenase followed by cytochrome *c* oxidase. Succinate dehydrogenase and NADH oxidase levels were low and could only be determined in the green stage of the fruit. However, peaks of various enzyme activities coincided in identical mitochondrial fractions on the sucrose density gradient. Moreover, the levels of malate dehydrogenase and cytochrome *c* oxidase were constant during the ripening process while the other two enzymes, succinate dehydrogenase and NADH oxidase, declined. This might indicate that mitochondria retain some of their essential functions through the ripening process.

INTRODUCTION

The phenomenon of fruit ripening is quite complex [1, 2]. The production of ripening hormones and other endogenous factors is affected by the environmental conditions [3] and the presence of specific enzymes. Baqui *et al.* [4] have investigated some mitochondrial enzymes, particularly Krebs cycle enzymes, and have indicated a significant increase in the activities of these enzymes in the ripening mango fruit. On the other hand, total mitochondrial number and size are reported to diminish during the ripening process [5, 6]. The present investigation was undertaken to study the behaviour of some enzymes in the purified mitochondrial fraction of tomato fruit.

RESULTS AND DISCUSSION

The mitochondrial fraction was isolated from 4 stages of ripening tomato fruit and purified on a sucrose density gradient. Four enzymes were assayed in the mitochondrial fraction.

Protein distribution curve

Protein concentration and malate dehydrogenase activity emerged in one gradient fraction. This was considered to be an indicator of the mitochondrial band. Malate dehydrogenase can be used for identification of mitochondria [6, 7], although this enzyme is not specific for the mitochondrial fraction.