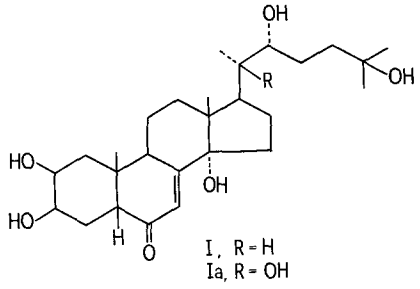
except that a pH 7.8 phosphate buffer was used[4]. Ecdysones were extracted and fractionated according to previously described methods[5], and extracts were analyzed by high-pressure liquid solid chromatography (HPLSC)[6]. The product 20-hydroxyecdysone was identified by comparative thin-layer chromatography (TLC) and HPLSC (Zorbax-Sil)[7] and by NMR and mass spectroscopy.



I, R = H
Ia, R = OH

Hornworm midgut ecdysone 20-hydroxylase showed the following properties: 1. An absolute requirement for NADPH; 2. A possible mitochondrial location based on activity present only in the 10,000 *g* pellet; and 3. Activity, based on our current findings, present only during the late 5th-larval and early prepupal stages. A number of the other tissues of the early prepupa was examined for enzyme activity and, based on TLC and HPLSC analyses, only the fat body possessed slight 20-hydroxylase activity. No activity was detected in the foregut, hindgut, Malpighian tubules, brain, or blood or in the ventral nerve cord. However, these results may in part reflect the titer of enzyme present in each tissue and the limitations of the analytical methods used. It is also possible that the time of appearance of ecdysone 20-hydroxylase in various tissues may differ from that in the midgut and thus it may not have been detected. The titer of the midgut 20-hydroxylase during the ages in which it is present is appreciable and the production of 25 µg of 20-hydroxyecdysone from 250 µg substrate by preparations from 20 prepupal midguts during the 4-hour incubation period was not uncommon.

The disappearance of the midgut 20-hydroxylase activity during the period between the prepupal and pupal stages of the hornworm is particularly striking. This enzymic activity is detectable until the time of appearance of 2 sclerotized bars on the dorsal thorax of the prepupa, which under the rearing regime used in our laboratory, typically indicates that the insect will molt to a pupa within 24 h. Moreover, midgut ecdysone 20-hydroxylase activity was found to be absent on days 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 of pupal life.

Other stages and ages examined and found to lack the ecdysone 20-hydroxylase activity in the midgut were: 24-hour-old 1st-, 2nd, 3rd, 4th and 5th-instar larvae and 24-hour-old adult males and females. In addition, the total homogenates of 24-hour-old eggs and of 1-day-old and 6-day-old pupae minus the midgut lacked the capacity to convert α-ecdysone to 20-hydroxyecdysone.

The results of the present in vitro studies are supported by in vivo studies that indicate the presence of appreciable quantities of 20-hydroxyecdysone in the 5th-instar larva[8] and the prepupa of the hornworm[9] and by in vitro studies that demonstrate the conversion of α-ecdysone to 20-hydroxyecdysone by tissues of the hornworm prepupa[10]; they do not provide information concerning either the biosynthesis or the source of the high titer of 20-hydroxyecdysone found in the pupa of this insect[9,11]. Interestingly, this enzyme is similar to mammalian cholesterol 20-α-hydroxylase with its activity dependent upon NADPH and with a possible mitochondrial location[12,13]. This similarity warrants further study, particularly with other substrates.

No inference can be made as to whether this enzyme produces 20-hydroxyecdysone from α-ecdysone for secretion into the blood or for the metamorphosis of the midgut[14] itself or for excretion[8]. Due to the sudden appearance and disappearance of midgut ecdysone 20-hydroxylase activity, the midgut should be investigated further in relation to the biochemical control of ecdysone 20-hydroxylase activity and as an assay organ for candidate insecticides designed for a hormonal mode of action.

[5] J. N. Kaplanis, M. J. Thompson, S. R. Dutky, W. E. Robbins and E. L. Lindquist, Steroids *20*, 105 (1972).
[6] H. N. Nigg, M. J. Thompson, J. N. Kaplanis, J. A. Svoboda and W. E. Robbins, Steroids *23*, 507 (1974).
[7] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.
[8] J. N. Kaplanis, M. J. Thompson, W. E. Robbins and E. L. Lindquist, Steroids *20*, 621 (1972).
[9] J. N. Kaplanis, M. J. Thompson, R. T. Yamamoto, W. E. Robbins and S. J. Louloudes, Steroids *8*, 605 (1966).
[10] D. S. King, Am. Zool. *12*, 343 (1972).
[11] J. N. Kaplanis, W. E. Robbins, M. J. Thompson and A. H. Baumhover, Science *166*, 1540 (1969).
[12] S. Ichii, S. Omata and S. Kobayashi, Biochim. biophys. Acta *139*, 308 (1967).
[13] G. Constantopoulos, P. S. Satoh and T. T. Tchen, Biochem. biophys. Res. Commun. *8*, 50 (1962).
[14] K. J. Judy and L. I. Gilbert, J. Morph. *131*, 277 (1970).

Inhibition of Prodigiosin Formation in *Serratia marcescens* by Adenosine Triphosphate

A. O. Lawanson and F. O. Sholeye

Biological Sciences Department, University of Ife, Ile-Ife (Nigeria), 15 September 1975.

Summary. ATP, inorganic phosphate and ribose inhibited prodigiosin formation in *Serratia marcescens*, but adenine did not. ATP was not hydrolyzed by the organism during the experiment.

Materials and methods. The Commonwealth mycological institute type collection number 89668 of *Serratia marcescens* was utilized. Cultures were maintained on liquid peptone-glycerol medium (pH 7.2) according to the procedure of Goldschmidt and Williams[1]. ATP, inorganic phosphate, ribose or adenine (0 to 1 µmole/ml), sterilized by filteration through membrane filters, was introduced into the culture medium immediately after inoculation.

[1] M. E. Goldschmidt and R. P. Williams, J. Bact. *96*, 609 (1968).

Table I. Inhibition of prodigiosin formation in *Serratia marcescens* by ATP and inorganic phosphate

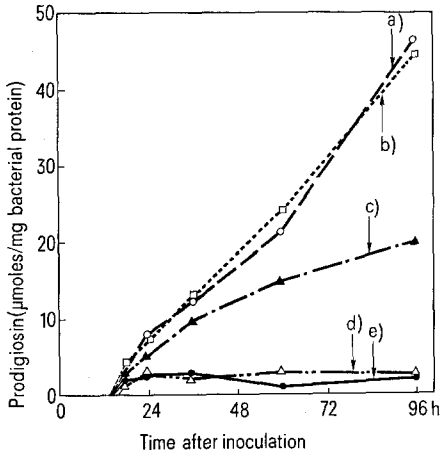
ATP (μmoles/ml)	Free inorganic phosphate in ATP (μmole/ml)	Inhibition (%)	
		Due to ATP (observed)	Due to P_i[a] (expected)
0	0	0	0
0.05	0.004	0	0
0.1	0.006	36.5 ± 0.4	4.4
0.5	0.03	76.7 ± 0.2	50.0
1.0	0.033	78.9 ± 0.2	53.0

Prodigiosin was determined after 48 h of growth. Concentration of prodigiosin in the control cultures was 13.8 μg/ml bacterial protein. Each value represents a mean of 10 determinations ± SD.
[a] P_i, inorganic phosphate.

Table II. Inhibition of prodigiosin formation in *Serratia marcescens* by ribose

Ribose (μmole/ml)	Prodigiosin formed (μg/mg bacterial protein)	Inhibition (%)
0	15.64	0
0.05	11.10	29.0 ± 0.1
0.1	11.15	28.7 ± 0.3
0.5	11.04	29.4 ± 0.3
1.0	11.05	29.4 ± 0.4

Prodigiosin was determined after 48 h of growth. Each value represents a mean of 10 determinations ± SD.



Effect of ATP on prodigiosin formation in *Serratia marcescens*. a) control (no ATP); b) 0.05 (μmole/ml ATP); c) 0.1; d) 0.5; e) 1.0. SD varied between 0.3 and 0.7.

After 48 h of growth in light (2000 lux), or at intervals indicated, prodigiosin was extracted and determined by the method of Goldschmidt and Williams[1]; inorganic phosphate, where indicated, was estimated by the method of CHEN et al.[2]; and protein was determined by the method of LOWRY et al.[3].

Results and discussion. When *Serratia marcescens* was grown in the presence of 0.1, 0.5 or 1.0 μmole/ml ATP, the rate of prodigiosin formation was retarded, and the magnitude of the retarding effect increased with increasing concentrations up to 0.5 μmole/ml ATP (Figure). While the prodigiosin content of the control cultures and those containing 0.1 μmole/ml ATP continued to rise throughout the 96-h duration of the experiment, 0.5 or 1 μmole/ml ATP kept the level of prodigiosin very low (5% to 20% of control) throughout the experiment; 0.05 μmole/ml ATP had no effect on prodigiosin formation. Such a retardation may be due, amongst other things, to endogenous levels of inorganic phosphate in ATP[1], ribose or adenine component of ATP, and/or additional inorganic phosphate resulting from ATP hydrolysis by the organism[4–7].

The effect of inorganic phosphate on prodigiosin formation was quantified. The amount of free inorganic phosphate present in ATP at the start of the experiment was estimated, and the percentage inhibition expected from this amount was compared with the percentage inhibition due to ATP after 48 h of growth. Table I shows that inorganic phosphate caused 26 to 32% less inhibition than ATP, at all concentrations studied. Additions of 0.1, 0.5 or 1.0 μmole/ml ribose caused about 29% inhibition in prodigiosin formation after 48 h of growth (Table II). This is approximately equal to the inhibition which is unaccounted for by inorganic phosphate.

Adenine at concentrations of 0.05, 0.1 or 0.5 μmole/ml had no effect on prodigiosin formation in *Serratia*. Also, within the 48-h duration of the experiment, there was no appreciable hydrolysis of ATP by *Serratia*. These results suggest that the inhibition of prodigiosin formation by ATP in *Serratia* is due to the endogenons level of inorganic phosphate and ribose content in ATP.

[2] P. S. CHEN, T. Y. TORIBARA and H. WARNER, Analyt. Chem. *28*, 1756 (1956).
[3] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. V. RANDALL, J. biol. Chem. *193*, 265 (1951).
[4] S. M. H. QUADRI and R. P. WILLIAMS, Appl. Microbiol. *23*, 704 (1972).
[5] S. M. LEWIS and W. A. CORPE, Appl. Microbiol. *12*, 13 (1964).
[6] M. I. BUNTING, C. F. ROBINOW and H. BUNTING, J. Bact. *58*, 114 (1949).
[7] J. L. BLIZZARD and G. E. PETERSON, J. Bact. *85*, 1136 (1963).