α-Amylase and its Release by Prostaglandin $F_{2\alpha}$ in Barley Endosperm Slices

3′5′ cyclic AMP (cyclic AMP) has been implicated[1,2] as an intermediate in the action of a number of mammalian hormones. It may also have a role in gibberellic acid triggered release of α-amylase during cereal grain germination[3,4]. It now appears that prostaglandins may be concerned in regulation of cyclic AMP levels in a variety of mammalian tissues. Most of the work reported has concentrated on the action of prostaglandins E_1 and E_2. In general they decrease cyclic AMP concentration in adipose tissue and increase it in most other tissues studied[5]. Prostaglandin $F_{2\alpha}$ has been reported to have no effect on adenyl cyclase in thymic lymphocytes[6] or intact uterus strips[7].

The present work indicates that prostaglandin $F_{2\alpha}$ can trigger release of α-amylase in barley endosperm slices. The barley used was *Hordeum vulgare* L. var. Maris Otter, dehusked by treatment with 50% H_2SO_4 and stored at room temperature. 2 mm endosperm slices in groups of 10 were incubated for 24 h at 25 °C with 4 ml of solution as indicated below. 1 ml *M* NaCl was added to the solutions before homogenizing in a Potter type homogenizer. The homogenates were left to stand for 1 h at room temperature before centrifuging (MSE bench centrifuge, 5 min, speed 10). α-amylase activity in the supernatant was assayed at 25 °C by the iodine-dextrin colour method of BRIGGS[8] and expressed in arbitrary units (AU) per 10 slices as described by DUFFUS[9].

Prostaglandins E_1 E_2 A_1 and $F_{2\alpha}$ prepared by Dr. J. E. PIKE were made available by the Upjohn Company, Kalamazoo, Michigan 49001. Prostaglandin $F_{2\alpha}$ supplied as the tromethamine salt was easily soluble in water. Solutions of prostaglandins E_1 E_2 and A_1 were prepared by dissolving 1 mg of prostaglandin in 0.1 ml of 95% ethanol and making up to 1.0 ml with sodium carbonate solution (0.2 mg/ml). The final pH was between 6 and 7.5.

The results show that prostaglandin $F_{2\alpha}$ at a concentration of $10^{-5}M$ can trigger α-amylase release in barley endosperm slices. The effect is small compared to that of gibberellic acid at a concentration of $10^{-5}M$ but is similar to that reported previously[3] for cyclic AMP at the same concentration. It does not appear to have an additive (or any) effect on the response to gibberellic acid. Prostaglandins E_1 E_2 and A_1 had no effect, either alone or in combination with gibberellic acid. A control experiment showed that no inhibitory effect on gibberellic acid triggered α-amylase release was observed with ethanolic sodium carbonate solution at the appropriate concentration. Polyunsaturated acids such as linolenic and linoleic acid, naturally occurring in mature barley seeds and thought to be precursors of prostaglandin in animals also had no effect.

The amount of α-amylase released appears to be finite and does not increase with long incubation. It may be suggested, therefore, that prostaglandin $F_{2\alpha}$ may bring about the release of a small amount of preformed α-amylase, possibly through the mediation of cyclic AMP.

Zusammenfassung. Prostaglandin $F_{2\alpha}$ kann die Freisetzung von α-Amylase im Endosperm von Gerste (*Hordeum vulgare* L.).

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Relative activities of α-amylase released by the action of gibberellic acid and prostaglandin $F_{2\alpha}$

Addition	α-amylase activity in AU/10 slices
Gibberellic acid ($10^{-5}M$)	0.34 ± 0.08 (14)[a]
Prostaglandin $F_{2\alpha}$ ($10^{-5}M$)	0.020 ± 0.013 (14)
Distilled water	< 0.0020 (14)
Prostaglandin E_1 ($10^{-5}M$)	< 0.0030 (4)
Prostaglandin E_2 ($10^{-5}M$)	< 0.0010 (2)
Prostaglandin A_1 ($10^{-5}M$)	< 0.0005 (2)

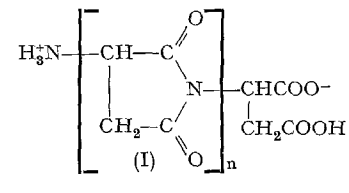
[a] The number of experiments is given in brackets.

1 E. W. SUTHERLAND, I. OYE and R. W. BUTCHER, Recent Prog. Horm. Res. *21*, 623 (1965).
2 G. A. ROBISON, J. Reprod. Fert. Suppl. *10*, 55 (1970).
3 C. M. DUFFUS and J. H. DUFFUS, Experientia *25*, 581 (1969).
4 A. G. GALSKY and J. A. LIPPINSCOTT, Pl. Cell Physiol. *10*, 607 (1969).
5 J. R. WEEKS, Naunyn Schmiedebergs Arch. Pharm. *269*, 347 (1971).
6 D. J. FRANKS, J. P. MCMANUS, J. F. WHITFIELD, Biochem. biophys. Res. Commun. *44*, 1177 (1971).
7 S. HARBON and H. CLAUSER, Biochem. biophys. Res. Commun. *44*, 1496 (1971).
8 D. E. BRIGGS, J. Inst. Brew. *67*, 427 (1961).
9 J. H. DUFFUS, J. Inst. Brew. *75*, 252 (1969).

The Hydrolysis of Polyimides[1]

Thermal polymerization of aspartic acid produces a polysuccinimide (I), a chain of aspartoyl residues[2,3]. We have investigated the alkaline hydrolysis of the imide rings of (I) which converts the polyimide to a polypeptide. The hydrolysis of imide rings has also interested investigators of the biological action of α-phthalimido-L-glutarimide (Thalidomide). The chemical reactivity of the phthalimide ring of Thalidomide has been established; for example, at pH 7 and 37 °C hydrolysis of the phthalimide ring proceeds at a significant rate[4].

The alkaline hydrolysis of polyimides can be expected to be kinetically complex due to increasing negative charge generated by carboxylate groups[5]. For this reason, a diimide, phthaloyl-DL-aspartoyl-β-alanine (IIA) was synthesized for a progressive study of the hydrolysis of polyimides. In addition, this diimide (IIA) can be related



(I)

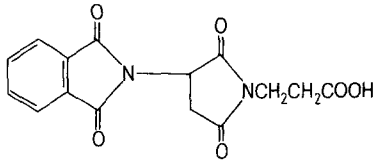
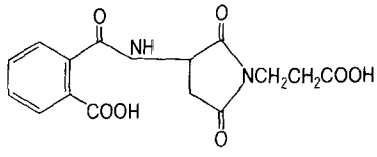
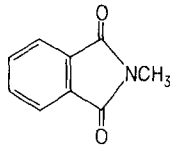
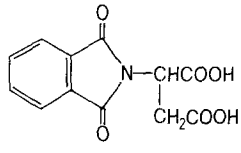
to Thalidomide and might be expected to exhibit similar reactivity during hydrolysis of the phthalimide ring. Phthaloyl-DL-aspartic anhydride was prepared by a method used for phthaloyl-DL-glutamic anhydride[6]. The former compound was fused with an equimolar amount of β-alanine at 190–200 °C for 30 min. The product was twice recrystallized from dimethylformamide and propanol-2, 233–235 °C mp; (theor. C, 56.96; H, 3.83, N, 8.86; found C, 56.86; H, 4.10, N, 8.88, Micro-Tech Laboratories, Inc., Skokie, Illinois).

Hydrolysis of phthaloyl-DL-aspartoyl-β-alanine was followed with a pH stat at 30 °C. At pH 7 one equivalent of base was required to neutralize the carboxyl group and another equivalent of base was consumed over 2 days in the hydrolysis of the phthalimide ring. This was established by a UV-spectrum which had features in common with those of both phthalyl-DL-aspartic acid and of succinoyl-β-alanine and which lacked the 220 nm absorption peak characteristic of the phthalimide ring[4]. A final equivalent of base was consumed during 12 h of hydrolysis of the aspartoyl residue at pH 9.5.

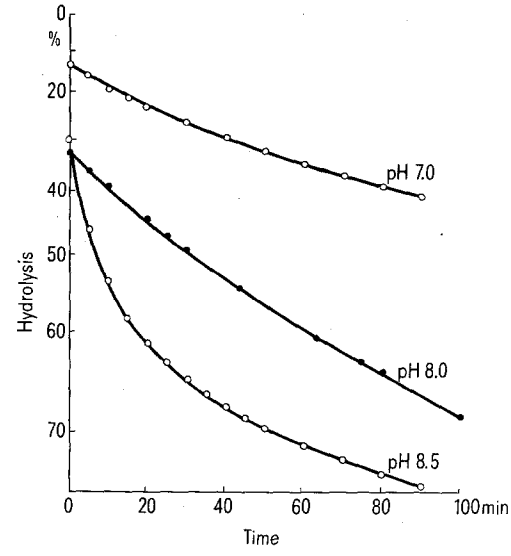
Reaction rates for the hydrolysis at 40 °C of a number of related imides are in the Table. Compared to the hydrolysis of N-methylphthalimide (III), an N-substituted succinimide ring increases the rate of hydrolysis of the phthalimide ring, whereas the N-substituted succinic acid of phthaloyl-DL-aspartic acid (IV) decreases the rate of hydrolysis. The succinimide ring can be expected to withdraw electrons from the phthalimide ring to increase the electrophilicity of the phthaloyl carbonyl carbon atoms, making them more attractive to hydroxide ions. The carboxylate groups of the succinic acid substituent present a large electrostatic repulsion to hydroxide ions and thereby decrease the rate of hydrolysis; in this case the hydrolysis is hydroxide ion concentration-controlled[7]. The two factors, induction and electrostatic charge, have thus been demonstrated to influence the rate of alkaline hydrolysis of polyimides.

Progress curves for the hydrolysis of the imide form of polyaspartic acid are shown in Figure 1. The initial observed pseudo first order rate of hydrolysis is high; it then decreases as hydrolysis proceeds. The high reactivity of intact imide polymer can be attributed to inductive activation of imide linkages by neighboring imide rings. In particular, the N-terminal succinimide ring should be very reactive because it is subject to electron withdrawal by the N-terminal amino group and by the N-penultimate imide ring. As hydrolysis of the polyimide proceeds, carboxylate groups are released and the electrostatic charge that is generated should repel hydroxide ions and thereby decrease the rate of hydrolysis. A similar effect of released carboxylate groups has been described for the alkaline hydrolysis of pectin[5]. The remaining imide rings in polyaspartic acid undergoing hydrolysis would be most resistant to alkaline hydrolysis due to the large negative charge on the polypeptide. The rate of imide hydrolysis at this point should be close to the rate of hydrolysis of phthalyl-DL-aspartoyl-β-alanine (IIB) (see

Observed second order rate constants for the hydrolysis of some related N-substituted phthalimides

Compound	K_{obs} (mole^{-1} min^{-1})[a]
(IIA) 	27,000 (for phthalimide ring
(IIB) 	643 (for succinimide ring)
(III) 	6,100
(IV) 	196

[a] 40 °C, 0.60 *M* KCl, rates determined at constant pH (P. D. Hoagland and S. W. Fox, J. Am. chem. Soc. *89*, 1389 (1967).



Progress curves for the hydrolysis of the imide form of polyaspartic acid at 40 °C in 0.60 *M* KCl at constant pH values af 7.0, 8.0, and 8.5. The plots deviate from first order kinetics.

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[2] J. Kovacs and I. Koenyves, Naturwissenschaften *41*, 333 (1954). – J. Kovacs, I. Koenyves and A. Pusztai, Esperientia *9*, 459 (1953).

[3] A. Vegotsky, K. Harada and S. W. Fox, J. Am. chem. Soc. *80*, 3361 (1958).

[4] S. Fabro, R. L. Smith and R. T. Williams, Nature, Lond. *208*, 1208 (1965).

[5] A. Katchalsky and J. Feitleson, J. Polymer Sci. *13*, 385 (1954).

[6] F. E. King and D. A. A. Kidd, J. chem. Soc. 3315 (1949).

[7] H. K. Hall jr., M. K. Brandt and R. M. Mason, J. Am. chem. Soc. *80*, 6420 (1958).

Table), which has two negative charges. Furthermore, the hydrolysis of imide linkages under mild alkaline conditions might be more selective in copolymers of aspartic acid, such as proteinoids[8]. Here the reactivity of the aspartoyl residues would be differentially influenced by the neighboring amino acid residue as well as by the number of free carboxylate groups. The esterase activity of a histidine-rich proteinoid has been found to be imide structure-dependent[9].

Imides are also of interest in other areas. A role for an aspartoyl group in an enzyme mechanism of action that involves active site acylation of an adjacent serine residue has been proposed[10,11]. Aspartoyl groups can be produced under certain relatively mild conditions during peptide synthesis[12,13].

Finally, the great reactivity of the phthalimide ring of phthaloyl-DL-aspartoyl-β-alanine is further evidence that the biological activity of Thalidomide resides in the acylating capability of its phthalimide ring[4]. Furthermore, we have been able to acylate methylamine, lysine, or glycine at pH 9.5 to 10.0 with the imide form of polyaspartic acid in water at room temperature. In these cases the amino groups compete with hydroxide ions for reaction with imide linkages. Coupling of amino compounds with the polyaspartic acid was significant, as judged by the following molar ratios calculated after exhaustive dialysis and amino acid analysis: 1. Methylamine:aspartic acid = 5:6; 2. Lysine:aspartic acid = 1:9; 3. Glycine:aspartic acid = 1:11. The reactivity of polyimides with amino compounds in water suggests that Thalidomide may interfere with embryo development through analogous acylation of amino groups of proteins, particularly histones.

Zusammenfassung. Nachweis, dass die alkalische Hydrolyse des Phthalimids durch eine N-Succinimidgruppe erhöht wird. Während der alkalischen Hydrolyse des Polysuccinimids fällt die Reaktionsgeschwindigkeit infolge Auftretens negativer Ladungen ab.

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17 January 1973.

[8] S. W. FOX and K. HARADA, J. Am. chem. Soc. *82*, 3745 (1960).
[9] D. L. ROHLFING and S. W. FOX, Archs Biochem. Biophys. *118*, 127 (1967).
[10] S. A. BERNHARD, A. BERGER, J. H. CARTER, E. KATCHALSKY, M. SELA and Y. SHALITIN, J. Am. chem. Soc. *84*, 2421 (1962).
[11] Y. SHALITIN and S. A. BERNHARD, J. Am. chem. Soc. *88*, 4711 (1966).
[12] D. F. DETAR, M. GOUGE, W. HONSBERG and U. HONSBERG, J. Am. chem. Soc. *89*, 988 (1967).
[13] D. F. DETAR and T. VAJDA, J. Am. chem. Soc. *89*, 998 (1967).
[14] Eastern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia (Pennsylvania 19118, USA).
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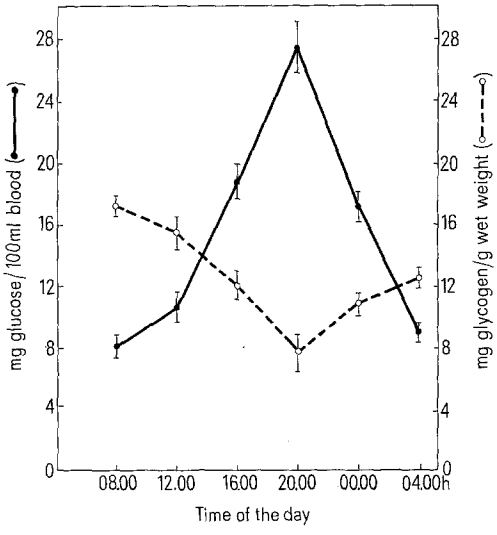
Circadian Rhythm in Blood Glucose and Liver Glycogen Levels of Scorpion, *Heterometrus fulvipes*

DEVARAJULU NAIDU[1] reported that the heart rate of scorpion, *Heterometrus fulvipes*, was shown to follow a regular circadian rhythm with the maximum rate at 20.00 h and the minimum at 08.00 h. The studies of VENKATACHARI and DEVARAJULU NAIDU[2] on choline esterase activity of the heart muscle of the scorpion, *Heterometrus fulvipes*, revealed that the maximum enzyme activity was with the maximum rate of heart beat and vice versa. Similar findings were also made on the rhythmic change of choline esterase activity in the ventral nerve cord of scorpion[3].



Circadian rhythm in relation to the levels of blood glucose and liver glycogen of Scorpion, *Heterometrus fulvipes*.

The above investigation suggests that the biological constituents (metabolites and enzymes) vary in a rhythmic manner during 24 h time. Among the metabolites that vary rhythmically are liver glycogen, blood glucose[4] and plasma free fatty acids[5]. But in the previous studies no attempt was made to analyse the rhythmicity of the metabolites like glycogen and glucose. This prompted us to study these constituents in scorpion blood and hepatopancreas (liver in chordates) at different intervals during 24 h day.

Material and methods. The commonly available South Indian Scorpion, *Heterometrus fulvipes*, was used during the present study. The animals were starved for 24 h preceding the estimations. The hepatopancreas (liver in chordates) were isolated at different times of the day in cold scorpion Ringer[6] and kept for 5 min for recovery. The blood from individual specimens was drawn with a

[1] V. DEVARAJULU NAIDU, Experientia *25*, 1274 (1969).
[2] S. A. T. VENKATACHARI and V. DEVARAJULU NAIDU, Experientia *25*, 821 (1969).
[3] S. A. T. VENKATACHARI and M. KRISHNA DASS, Life Sci. *7*, 617 (1968).
[4] A. SOLIBERGER, Ann. N.Y. Acad. Sci. *117*, 519 (1964).
[5] A. M. BARRETT, Br. J. Pharmac. *22*, 577 (1964).
[6] B. PADMANABHA NAIDU, Nature, Lond. *213*, 410 (1967).