Relative activities of digestive enzymes in marine molluscs of Lanzarote

Mollusc	α-Am.[a]	Cell.[b]	Lam.[c]	A.P.[d]	A.E.[e]
Bittium reticulatum	+	?	–	–	
Cantharidus exasperatus	+	+	?	–	
Cerithium vulgatum	+++		–	+++	++
Conus betulinus	+	++	?	++	–
Conus mediterraneus	–	++	++	++++	
Haliotis tuberculata	+++	+++	++++	++	–
Littorina striata	++	+++	++	?	–
Monodonta turbinata	+	++	++	?	–
Pusia tricolor	++	+	–	+	–
Rissoa costulata	++	++	++	–	–
Thais haemastoma	++	–	++	+++	–
Tricolia pullus	+++	++	+	+	–
Turbo rugosum	++++	++++	+	++	?
Chiton canariensis	++	+++	++	++	
Arca lactea	++	+	–		
Pinna rudis	+++		++	++	–
Aplysia ocellata	+++	+	?	+	
Polycera webbii	+		–	++	–

+, Trace activity; ++, moderate activity; +++, high activity; ++++, very high activity; –, no activity; ?, slight activity within margin of error.

Value of symbols above

Symbol	α-AM.[a]	Cell.[b]	Lam.[c]	A.P.[d] + A.E.[e]
+	0–5	0–0.5	0–0.05	0–0.5
++	5–15	0.5–2.0	0.05–0.20	0.5–1.0
+++	15–25	2.0–4.0	0.20–0.40	1.0–1.5
++++	Above 25	Above 4.0	Above 0.40	Above 1.5

[a] α-Amylase (IDC units/g fresh weight). [b] Carboxymethyl cellulase (Δ ε sp/g fresh weight). [c] Laminarinase (OD Units/g fresh weight). [d] Acid Phosphatase (OD units/g fresh weight). [e] Acid Esterase (OD units/g fresh weight).

All enzyme assays were carried out at 26 °C α-amylase[3], laminarinase[4], acid phosphatase[5] and acid esterase[5] were assayed by standard techniques. Cellulase activity was determined by a simplified viscometric technique. To 4 ml of 0.16% carboxymethyl cellulose was added 2 ml of enzyme extract, diluted with water where necessary. After shaking, 5 ml of the mixture was transferred to a U-tube viscometer and the viscosity measured at 10 min intervals. The rate of fall in specific viscosity gives a measure of the enzyme activity.

From the results (Table) it can be seen that all species studied except *Conus mediterraneus* have α-amylase activity. Cellulase too is present in all species with one exception, *Thais haemastoma*. This may be a reflection of the carnivorous habit of this particular species. Laminarinase is not nearly so widespread but it is at present impossible to place any satisfactory interpretation on its distribution. The same applies to acid phosphatase. The presence of acid esterase in *Cerithium vulgatum* in quantity appears to be unique. Again it is difficult to correlate this with its feeding habit but it may be a fact of some taxonomic interest[6].

Résumé. Les quantités de α-amylase, laminarinase, cellulase, acide phosphatase et acide esterase ont été estimées par l'examen des extraits de dix-huit expèces de mollusques marins récoltés dans l'île canarienne de Lanzarote.

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Deamination by 'Transformed' Mitochondrial Amine Oxidase of the ε-Amino Group of Lysine and Selective Inhibition of this Reaction by β-Aminopropionitrile

Although the significance for desmosine and isodesmosine biosynthesis of deamination of the ε-amino group of lysine is well recognized[1] enzymes catalyzing this reaction in animal tissues are still unknown[2]. There are no data on deamination of lysine by pig plasma amine oxidase[3]; a closely related enzyme – beef plasma amine oxidase – does not catalyse any measurable oxidation of lysine[4].

A phenomenon operationally defined as 'transformation' of mitochondrial monoamine oxidase into an enzyme resembling diamine oxidases (including those of plant origin which do oxidize lysine[5]) is observed in vitro in liver mitochondria treated under certain conditions by oxidized oleic acid[6,7] and is also possible in vivo[8].

If a hypothesis[7] on participation in cross-link formation and in pathogenesis of experimental lathyrism of the 'transformed' mitochondrial amine oxidase deserves further consideration, one would expect that the enzyme will deaminate ε-amino group of lysine and that this reaction will be inhibited selectively by low concentrations of a potent lathyrogen-β-aminopropionitrile[9].

Methods used for isolation of rat and beef liver mitochondria, preparation of 'transformed' mitochondrial amine oxidase, assay of amine oxidase activity were described previously[6,7,10].

The data presented in Table I suggest that while L-lysine and its derivatives possessing substituted α-amino group are readily deaminated by 'transformed' mitochondrial amine oxidase (with rates comparable to those found in experiments with other ω-amino acids or amines), substitution of ε-amino group of L-lysine prevents its

Table I. Deamination of some amino acids and amines on their incubation with 'transformed' beef liver mitochondrial amine oxidase.

Substrates and their concentrations	$M \times 10^{-3}$	V_{max} values (mμmoles of NH_3/mg of protein/min) mean values ± S.E.	
Histamine · HCl	10	2.8 ± 0.6	(3)
Histidine · HCl	10	0	(4)
Cadaverine · 2 HCl	10	2.5 ± 0.2	(4)
L-Lysine · HCl	15	1.5 ± 0.07	(8)
α-Hippuril-L-lysine	10	2.2 ± 0.1	(3)
α-Carbobenzoxy-lysine[11]	10	0.95, 0.47	(2)
ε-Carbobenzoxy-lysine[11]	10	0 0	(2)
L-Ornithine	5	1.7 ± 0.1	(4)
γ-Aminobutyric acid	20	1.9 ± 0.4	(5)
α-Aminobutyric acid	10	0	(4)
β-Alanine	10	1.3 ± 0.2	(5)
α-Alanine	10	0	(3)

Composition of samples and experimental conditions as described[6]. Number of experiments in brackets.

Table II. Effect of β-aminopropionitrile fumarate on the activity of 'transformed' rat liver mitochondrial amine oxidase.

Concentrations of the inhibitor ($M \times 10^{-3}$)	Inhibition of deamination (%) (mean values from the data of 4–6 parallel assays)		
	lysine	serotonin	γ-ABA
1.0	100	9.5	–
0.1	100	11.5	5.0
0.01	45.5	–	12.0

Composition of samples as described[6]. The inhibitor[12] was preincubated at room temperature with the enzyme preparations for 30 min before the addition of one of the substrates in following concentrations ($M \times 10^{-3}$): serotonin creatinine sulphate (5), DL-lysine·HCl (15), γ-aminobutyric acid (ABA) (20); in control samples (without the inhibitor) deamination rates were respectively: 2.47, 1.71, 0.99 mμmoles of NH_3mg of protein/min.

deamination. Deamination of lysine on incubation with 'transformed' mitochondrial amine oxidase is completely inhibited by $10^{-4}M$ β-aminopropionitrile while its inhibitory effect on deamination of other substrates of the enzyme (serotonin and γ-aminobutyric acid) is negligible (Table II).

It is noteworthy that the activity of 'transformed' mitochondrial amine oxidase is not inhibited by high concentrations of conventional monoamine oxidase inhibitors[6] which do not produce experimental lathyrism[13].

Выводы. «Трансформированная» митохондриальная аминоксидаза катализирует отщепление ε-аминогруппы лизина. Эту реакцию избирательно тормозит латирогенный агент β-аминопропионитрил.

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Interconversion of Cortisol and Cortisone in the *Macaca mulatta*

The principle corticosteroids present in mammalian blood are cortisol, corticosterone and aldosterone[1–7]. Although small amounts of 11-desoxy-17α-hydroxycorticosterone (pregn-4-ene-17α, 21-diol-3-one), 11-dehydrocorticosterone (pregn-4-ene-21-ol-3, 11-dione), cortisone (pregn-4-ene-17α, 21-diol-3, 11-dione), and 11-desoxycorticosterone (pregn-4-ene-21-ol-3-one) have been detected in blood, their concentrations in blood have not been established[8,9]. Of these steroids, cortisol is considered to be the physiologically active glucocorticoid in man. The efficacy of cortisol in affecting thymus involution, liver glycogen deposition and anti-inflammatory response exceeds that of cortisone[10]. Furthermore, cortisol in vitro markedly inhibited the glucose uptake by rat thymus cell suspension while cortisone was ineffective[11]. Since the 11β-hydroxysteroid dehydrogenase activity is low in the thymus[12], this finding supports the proposition that cortisol is the active agent and the biological efficacy of cortisone is dependent upon its conversion to cortisol. On the other hand, KOIKE et al.[13] demonstrated that the protein synthetic activity of polysomes from livers of cortisone-treated rats was greater than that of cortisol-treated rats. These seemingly divergent results suggest that cortisol and cortisone may be active depending on the tissue or assay systems used, or that a balance of these steroids may be of importance for their biological efficacy. This study was conducted to observe the interconversion of these 2 steroids in vivo in the *Macaca mulatta* and to establish the relative levels of these steroids in blood.

Material and methods. 1, 2-^{3}H-cortisol and 1, 2-^{3}H-cortisone with specific activities of 22 and 36 c/mmole were