REVISTA ESPAÑOLA DE FISIOLOGIA, 29, 217-222. 1973

Two Different Amylase Activities in the Sea Mussel, *Mytilus edulis* L. *

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(Received on May 15, 1973)

M. ALEMANY and M. ROSELL-PEREZ. *Two Different Amylase Activities in the Sea Mussel,* Mytilus edulis *L.* Rev. esp. Fisiol., 29, 217-222. 1973.

Digestive β-amylase activity has been previously described in the sea mussel. It has been observed that in this animal species there are two different amylase activities, both of them α-amylase type. These enzyme activities show different optimum temperature and *p*H behavior, and are differently activated by effectors, and precipitated with different concentrations of neutral salts. The two enzyme activities show an almost ubiquitous distribution in all tissues and organs, with appreciably high levels of activity. These activities are also clearly found in non-digestive organs as gonadal tissues, and, especially, in the muscle tissue.

It is suggested that the amylases found can play a complementary role in the rapid mobilization of glucidic fuels during the physiological anoxic cyclic states of this animal species.

While studying the glycogen metabolism in the sea mussel, we observed the occurrence of very high levels of amylase activity, directly responsible for the high amount of oligosaccharides (7) present in the animal's tissues. This amylase activity was already described as digestive, because of its limited distribution in the digestive cristalline needle formation that opens into the stomach. This amylase was formerly described as a β-amylase (2, 15) because of its *p*H requirements and by the kind of mild dextrinification and high sugar-producing hydrolisis that it causes on amylose chains (6).

A similar amylase activity was early observed in the digestive gland, and also in gonadal tissue homogenates. This activity is very noticeable in animals that suffer anoxia, and yields a rapid increase in the levels of reducing sugars, producing a faster decrease in the glycogen content of the tissues.

* A preliminary report about this paper was presented at the XI Jornadas Bioquímicas Latinas, Salamanca, April 25-28, 1973.

** Supported by a «Beca de formación de Personal investigador» and «Ayuda a la Investigación» from the Ministerio de Educación y Ciencia of Spain.

It is suggested that this activity can be, to some extent, responsible for the deep and rapid mobilization of the glucidic fuels, stored as glycogen in almost all tissues in the animal, with the purpose of coping with the energy requirements of the animal under anoxic conditions.

Materials and Methods

The mussels were commercial ones, belonging to the species *Mytilus edulis* L., coming from the NW of Spain. They underwent, in the transport process, some degree of anoxia, but all the animals used in the experiments were completely alive and normal, by a standard macroscopic examination.

For the estimation of the levels of amylase activity, the tissues were homogenized (1:10 w/v) in cold 0.1 M tris(hydroxymethyl)-aminomethane-HCl buffer, *p*H 7.0, and afterwards the following assay mixture was incubated 5-20 minutes at 30° C (25° C): 0.05 ml of the homogenate, 0.2 ml of 0.2 M phosphate buffer, *p*H 7.0 (or 0.2 ml of 0.2 M acetate buffer, *p*H 5.0), and 0.1 ml of 1 % soluble starch. The reaction was started by the addition of the homogenate, and was stopped by adding 0.5 ml of ice cold 0.2 N HCl. The color was developed immediately with 10 ml of 0.1 % KI with 0.5 mM KIO_3, and was read against water in a spectrophotometer at 625 nm (11).

Some determinations were also conducted with a reductometric method (1) based upon the reduction of alkaline 3,5-dinitrosalycilic acid, that depends on the liberation of reducing sugars from native mussel's glycogen. There were not significative differences between the results of the two procedures except that, due to the large amounts of reducing sugars already present in the tissues, the sensitivity of the latter method was lower, and we preferred to use the amyloclastic one for all the following determinations.

The chromatographic separation of sugars was achieved by thin layer chromatography, with Silicagel G plates or with kieselguhr ones (14), 0.25 mm thick, and also by paper chromatography using paper «Whatman» nr. 1. The eluting mixtures utilized were isopropanol/acetic acid/water (3:1:1) (1), *n*-butanol/ethanol/water (40:11:19) (10) and methanol/acetone/water (4:5:1) (4). The color-developing reagents utilized were saturated $AgNO_3$ solution in acetone (10) for the paper chromatography, and the anisaldehyde-sulphuric acid reaction from STAHL and KALTENBACH (12) for the thin-layer chromatography.

All reagents utilized were analytical grade, proceeding from «Merck», «Fluka», «Carlo Erba» and «Panreac».

Results

Initially we confirmed chromatographically the results of FRAGA (7) and others (8) about the high oligossacharide content in the tissues of the anoxic mussel. It was observed that the amylase activity of the crystalline needle formation yields, when incubated with mussel's glycogen, glucose and maltooligosaccharides (maltose, maltotriose and higher sugars) which means that these are enzymes belonging to the α-amylase type.

Simultaneously there was found in the digestive gland, and in the gonadal eminence, a stronger α-amylase activity, yielding higher amounts of heavy maltooligosaccharides but also maltose and glucose. The occurrence of very high amounts of glucose (more than 1 % of the fresh weight in the digestive gland) was attributed partially to a maltase activity, yielding glucose from maltose, which activity was also found by us.

The study of the *p*H-dependence of the amylase activity from the different tissues of the animal seems to demonstrate the occurrence of two different amylase activities in all tissues studied. It can be observed in figure 1 that the α-amylase acti-

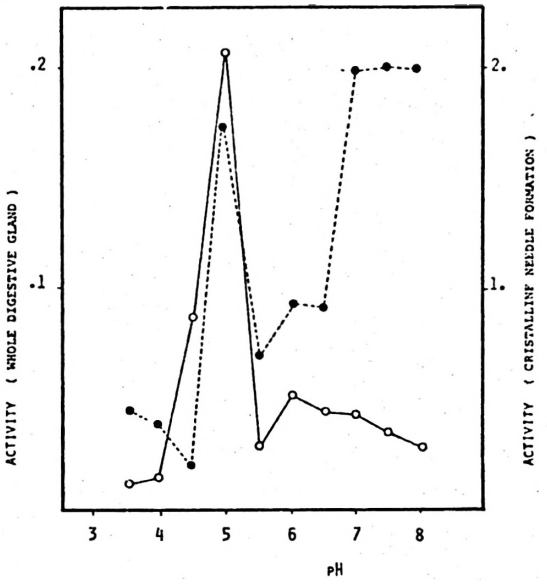

Fig. 1. *pH-dependent curves of activity for the amylase systems of the sea mussel.*
Crystalline needle formation: (○) and whole digestive gland: (●). The activities are expressed as mg of starch hydrolised by minute and g. of fresh tissue.

vity of the crystalline needle formation — acid amylase — is high in a narrow limit with a *p*H of 4.9 as optimum value, whereas that of the digestive gland homogenates shows a similar peak in the same place and a second maximum in the neutral-basic zone of the *p*H scale, with an optimum plateau of *p*H 7.0 to 8.0, corresponding to a neutral amylase.

Similarly, the temperature behavior of these enzyme activities seems to indicate (Figure 2) that the two activities are different, as the acid amylase activity has an optimum value near 25° C and the neutral amylase activity at about 32° C.

The ammonium sulfate fractionation of the homogenates (Table I) shows also the differences for both activities. The acid amylase activity could be recovered mainly with a 40 % saturation in the supernatant, whereas the neutral amylase was recovered mainly with a 50 % saturation.

The acid amylase activity was clearly activated by 50 mM calcium chloride and 50 mM sodium tetraborate, and resulted strongly inhibited by 50 mM sodium citrate, glycerol and ammonium sulfate (Table II). The alkaline halides (sodium and potassium chlorides, sodium bromide and sodium fluoride) showed some activating effect that decreases in the order listed. The neutral amylase was only slightly activated by 50 mM calcium chloride and was deeply inhibited by 50 mM sodium citrate, sodium tetraborate and ammonium sulfate. The magnesium acetate had a considerable inhibiting action upon the neutral amylase and a not so significant one upon the acid amylase. Therefore the re-

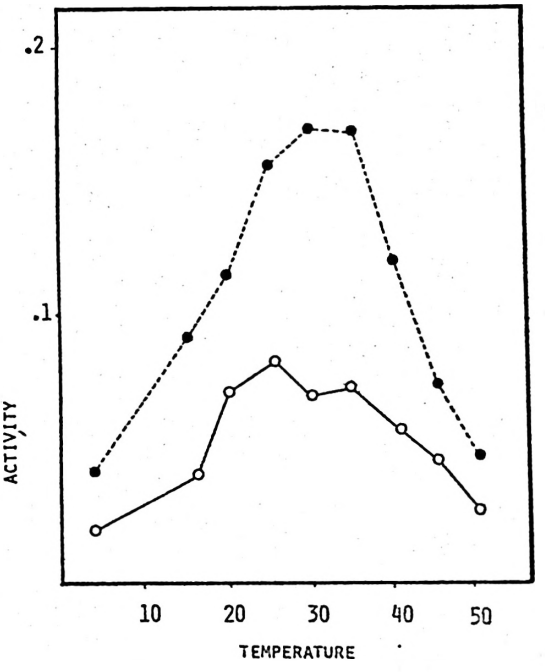

Fig. 2. *Temperature-dependent curves of activity for the amylases from the digestive gland of the sea mussel.*
Amylase activity determined at *p*H 7.0 (neutral amylase): (●); and amylase activity determined at *p*H 5.0 (acid amylase): (○). The activity is expressed as mg of starch hydrolised by minute and g. of fresh tissue.

Table I. *Percent of total activity recovered in the precipitates in ammonium sulfate fractioning of the sea mussel amylase systems.*

The results were obtained from single digestive gland homogenates to which sufficient saturated ammonium sulfate was added to obtain the desired concentration. After low speed centrifugation the precipitates were resuspended in distilled water for the estimation of the amylase activities. The supernatants were supplemented with more saturated ammonium sulfate to repeat the process.

	PERCENT OF SATURATION OF AMMONIUM SULFATE IN THE SUPERNATANT							
	10	20	30	40	50	60	70	80
Neutral amylase	9.8	13.8	11.2	17.8	21.8	16.7	8.2	0.7
Acid amylase	9.2	13.6	10.5	22.8	18.9	10.6	9.6	4.8

Table II. *Action of some effectors on the amylase activities of the sea mussel.*

Effector added [50 mM]	Acid amylase %	Neutral amylase %
None	100.0 *	100.0
Calcium chloride	165.0	101.0
Sodium tetraborate	134.5	71.2
Sodium chloride	122.0	97.7
Potassium chloride	120.0	97.5
Sodium bromide	109.2	96.8
Sodium fluoride	102.3	89.1
Magnessium acetate	84.3	54.8
Glycerol	79.9	96.5
Ammonium sulfate	74.1	87.0
Sodium citrate	61.0	50.9

* Percent of the normal activity yielded by the amylases in the presence of the compounds listed.

sults of activation-inhibition produced by some effectors on these two activities did not coincide.

The data listed in Table 2 give a correlation coefficient of 0.524 between the two columns, which means that the similarity of behavior of the effectors upon these two activities is not significant ($p < 0.1$).

The specific activity of the two amylase types of activity was also determined and the data obtained are given in Table 3.

The relationship between the specific activities of the two amylases in different organs is fairly constant. It is roughly 1 in all studied organs, with the exception of the crystalline needle formation, which has practically only acid amylase, and the digestive gland, in which the mean ratio of amylase activities acid/neutral is 0.55.

Table III. *Distribution of the two amylase activities in different parts of the sea mussel.*

ORGAN	SPECIFIC ACTIVITIES *	
	Acid amylase	Neutral amylase
Digestive gland (complete)	12.8 ± 6.93 **	20.7 ± 3.32
Crystalline needle formation	128.0 ± 7.31	—
Mantle	0.4 ± 0.28	0.5 ± 0.49
Main abductor muscle	3.5 ± 0.21	3.5 ± 0.27
Gonadal eminence	2.0 ± 1.23	2.0 ± 1.90

* The specific activities are expressed as mg of starch hydrolised by gram of fresh tissue and minute of incubation at the optimum temperature and pH.
** Mean ± s. d.

Discussion

The high levels of amylase activity found in the sea mussel could be considered as digestive if referred only to the digestive organs and related structures, but it is not, obviously, a sufficient explanation after finding patent amylase activities in such organs as the main abductor muscle, unquestionably unrelated with digestion.

After these suggestive facts, it was found that the amylase activity of the tissues studied was actually the added activity of two enzymes, the acid amylase, found in all studied tissues — and especially in the crystalline needle formation —, and the neutral amylase, ubiquitous as the other with the exception of the crystalline needle formation.

These two enzyme activities seem to correspond to two really different enzymes, as shown by their different *p*H — and temperature — dependent behavior, and also by their significant difference in recovery by precipitation with ammonium sulfate, and activation-inhibition patterns with different effectors. Their activation by calcium ions is very significant, and results markedly manifested on the acid amylase. It must be taken into account that calcium plays a very important role in this animal species as an alkaline reserve for minimizing the acidosis produced by anoxia (5, 3); and these animals were actually suffering anoxia to some degree. This signifies that the calcium concentration in blood and body fluids could have been sufficiently high to activate, to some extent, the amylase activity. In this respect we suppose that the neutral amylase can be completely activated with these minimum amounts of calcium — as demonstrated by the dramatic lowering of the activity in presence of 50 mM sodium citrate, an effective calcium-removing agent —, whereas the acid amylase would require higher levels of this cation in order to be activated.

The specific activities of the two amylases in various organs of these animals show an apreciable individual dispersion, but with a relative parallelism between all the organs of the same animal. There are animals with «low» amylases and others with «high» ones. There is also a characteristic ratio of activities between the two amylases for every organ, which is frequently near 1, except for the digestive organs. It is difficult to imagine what is the real role of the acid amylase in the animal's tissues because of its *p*H requirements, which are too acid. The neutral amylase can be considered as part of the emergency degradative systems for the rapid mobilization of glucose units from glycogen. Tentatively this hypothesis is suggested as a possible explanation because of the lability of the glycogen-phosphorylase in this animal and its requirements in order to exert its action (9, 13). It is supposed that the normal glycogen degradation can be carried out by the phosphorylase and the emergency rapid mobilization of sugars can be due to the amylase systems.

Resumen

En el mejillón había sido ya descrita previamente la existencia de una actividad β-amilásica de índole digestiva; en este trabajo se ha podido comprobar que en dicha especie coexisten dos actividades amilásicas distintas, ambas pertenecientes al tipo α-amilasa. Dichas actividades muestran comportamientos diferentes frente al *p*H y a la temperatura de incubación, resultando asimismo afectadas de distinto modo por la adición de efectores, precipitando también con concentraciones diferentes de sales neutras.

Ambas actividades enzimáticas muestran una distribución casi ubicua en todos los órganos y tejidos estudiados del animal, con niveles de actividad apreciablemente altos.

Ambas actividades amilásicas se encuentran en órganos de naturaleza no digestiva como el tejido gonadal y, especialmente, el tejido muscular.

A la vista de esta evidencia se sugiere la

posibilidad de que las amilasas jueguen un papel complementario en la movilización rápida de material glucídico en las fases fisiológicas cíclicas de anoxia por las que dichos animales atraviesan normalmente.

References

1. CLARK, J. M. (Editor): *Experimental Biochemistry*, 1964, Freeman & Co., San Fran- and London, 1964.
2. COE, W. R.: *J. Exptl. Zool.*, **99**, 1, 1945.
3. CULBRETH, S. E.: *Biol. Bull.*, **80**, 79, 1941.
4. DAWSON, R. M. C., ELLIOTT, D. C., ELLIOTT, W. H. and JONES, K. M. (Editors): *Data for Biochemical Research*, Oxford University Press, Oxford, 1969.
5. DUGAL, L. P.: *Journ. Cell. Comp. Physiol.*, **13**, 235, 1939.
6. FOX, D. L. and MARKS, G. W.: *Bull. Scrips. Inst. Oceanog.*, **4**, 29, 1936.
7. FRAGA, F.: *Invest. Pesquera*, **14**, 25, 1958.
8. FRAGA, F. and LÓPEZ-CAPONT, M.: *Invest. Pesquera*, **11**, 39, 1958.
9. GARCÍA-FERNÁNDEZ, M. C. and ROSELL-PÉREZ, M.: *Res. Comunic. XII Reunión S.E.B.*, Madrid, 1972.
10. RANDERATH, K.: *Cromatografía de capa fina*, Ed. Urmo, Bilbao, 1969.
11. SOMOGYI, M.: *Clin. Chem.*, **6**, 23, 1934.
12. STAHL, E. and KALTENBACH, U.: *J. Chromatog.*, **5**, 351, 1961.
13. VÁZQUEZ, M. I., GARCÍA-FERNÁNDEZ, M. C. and ROSELL-PÉREZ, M.: *Res. XI Jornadas Bioquímicas Latinas*, Salamanca, 1973.
14. WEILL, C. E. and HANKE, P.: *Analyt. Chem.*, **34**, 1736, 1962.
15. YONGE, C. M.: *Sci. Progress*, **26**, 643, 1932.