ANNAL. BIOL. TIHANY | 36 | 223—228 | HUNGARIA 1969

ON SOME PROPERTIES OF THE EXOPEPTIDASE OF *GAMMARUS (RIVULOGAMMARUS) ROESELI* GERVAIS (AMPHIPODA) AND *ASELLUS AQATICUS* (L.) (ISOPODA)

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Received: 7th February, 1969

Earlier investigations (KLEINE and PONYI, 1967; KLEINE, 1967; DEVILLEZ, 1965) revealed that the stomach juice and the hepatopancreas of some Decapod species contain a carboxypeptidase with highly similar effects to the pancreatic carboxypeptidase of the mammals, though exhibiting but meagre substrate specificity.

Within the class Crustacea, there are no data on the exopeptidase conditions of groups phylogenetically removed from the Decapods. The present paper proposes to discuss some properties of the carboxypeptidase of one representative each of the orders Amphipoda and Isopoda.

Material and method

1. *Material under investigation and the preparation of the ferment extract*

The animals used in our investigations *(Gammarus, Asellus)* had been collected from one of the tributary streams (Aszófői patak) of Lake Balaton, and kept, for at least 2 weeks prior to their preparation, in a well aerated aquarium supplied with through-flow water system.

The ferment extract was taken from the intestinal tract of the animals; the dissecting technique has already been published in an earlier paper (PONYI and P.-ZÁNKAI, 1967). The intestinal tract of 50 *Asellus* and 25 *Gammarus* specimens was used per each experimental series. The prepared organs were collected in 2 ml distilled water and immediately placed in ice or in refrigerators, respectively, and kept there, except for the period of centrifuging, until their employment. The material was homogenised by Potter's glass homogenisator. Centrifuging took 10 minutes, at 5000 r.p.m.

The stomach and the stomach juice of *Gammarus* and *Asellus*, as well as the midgut, are infinitesimally smaller in comparison to the relatively large hepatopancreatic (HP) tubes and to the liquid content in them. Thus the extract obtained from the animals originates mainly from the HP tubes and from their contents.

2. *Substrate incubation conditions methods of identification*

Two peptide substrates have been used in our investigations: carbobenzoxy-L-glutamyl-L-tyrosine (CGT) (pepsine and carboxypeptidase-A-homospecific substrate) (Sas et Son Ltd.), and carbobenzoxyglycyl-L-phenylalanine (CGP) (carboxypeptidase-A-specific substrate) (Fluka).

The substrate concentration in 1 ml of reaction mixture is shown in *Table 1.* The composition of the incubational mixture is as follows:

	Enzyme	Asellus substrate ml	Buffer	Enzyme	Gammarus substrate ml	Buffer
CGT	0.08	0.12	0.80	0.03	0.10	0.87
CGP	0.08	0.10	0.82	0.02	0.05	0.93

The buffers employed were: McIlvaine (0.1 M; between pH 3.1—6.5), tris (HCl) 0.1 M; between pH 7.2—9.1). The homogenizates were incubated for 30 min at 37.5 $\pm$ 02. °C. The value of the control was 0 for both species.

During the quantitative paper chromatography (Hanson, 1966), the ninhydrin-copper complex was measured with Beckman spectrophotometer at 495 $\mu\mu$. The protein content of the enzyme preparation was determined by

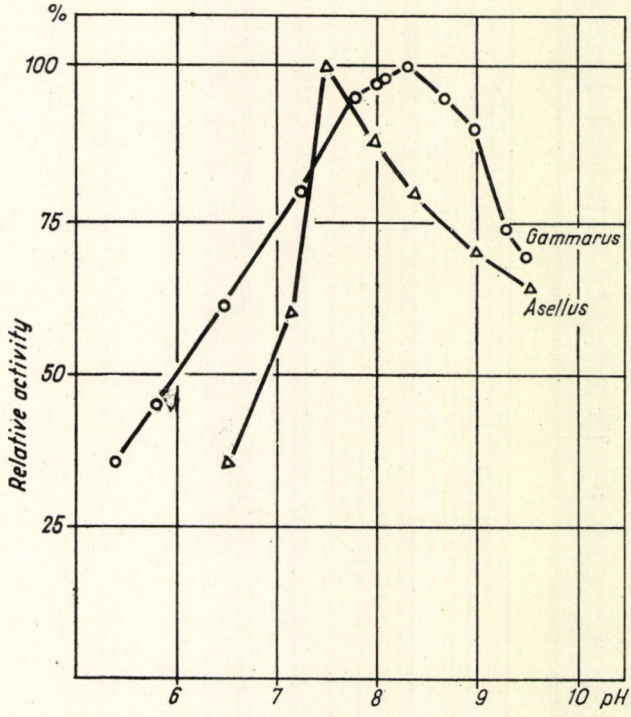

Fig. 1. The pH activity curve, with respect to CGP, of the ferment extract of *Gammarus (Rivulogammarus) roeseli* Gervais and *Asellus aquaticus* (L.)

the LOWRY method modified by GLÄSSER and KLEINE (1962). Crystallized bovine serum-albumen (Albumine bovine (fraction V) B grade (Calbiochem)) was used for standard. The albumen content of the *Gammarus* and *Asellus* enzyme perparations varied between 2.5—6.4 mg/ml and 3.6—6.2 mg/ml, respectively.

During the preliminary investigations, we used a freshly prepared sephadex-G-100 column, set at 7.5 pH by a 0.05 phosphate buffer. The size of the tube was 1.5 × 24 cm. The fractions were collected by a home-made automatic microfraction collector built in a refrigerator. 1.25 ml fractions were collected.

Results and discussion

For the demonstration and characterization of the carboxypeptidase-A effect CGP was used. The pH optimum of the clevage in *Asellus* (between 7.5—7.7) agrees with that of *Astacus* and *Cambarus*, but rather deviating (between 8.0—8.2) in *Gammarus (Fig. 1)*. CGT was applied to show the eventual pepsin or cathepsin-A effect. The pH optimum is identical (7.2 pH; *Fig. 2.)* for both species and agrees with the conditions found in the two Decapod species studied earlier; the presence of a carboxypeptidase is also obvious here (KLEINE and PONYI, 1967).

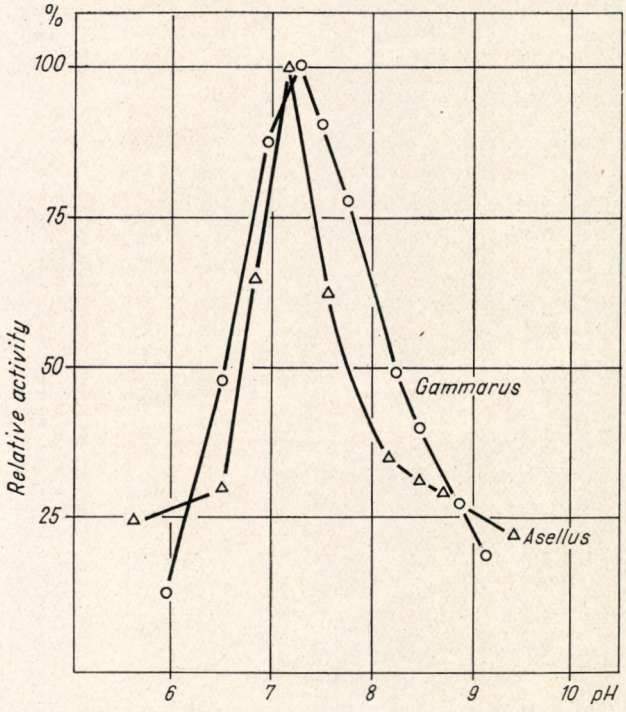

Fig. 2. The pH activity curve, with respect to CGT, of the ferment extract of *Gammarus (Rivulogammarus) roeseli* GERVAIS and *Asellus aquaticus* (L.)

The quotient of CGP/CGT activity of *Asellus* and *Gammarus* points to the same small substrate specificity as was observed also in the case of *Astacus* and *Cambarus:*

Astacus	1,01
Gammarus	1.23
Asellus	1.28
Cambarus	1.42

The CGP/CGT quotient is 8.3 in the case of the mammalian pancreas (HOFMAN and BERGMANN, 1940).

The specific activity of the *Gammarus* and *Asellus* carboxypeptidase (v_s) significantly differs, according to the investigations hitherto conducted, from that of the Decapods. Whereas this value concerning the HP of *Astacus* is 2.00 (CGP) and 1.64 (CGT), respectively, and for the stomach juice 5.21 (CGP) and 5.15 (CGT), respectively, the figures are about ten times higher for the species investigated. *(Table 1).*

Table 1

The exopeptidase activity of the hepatopancreas of *Gammarus (Rivulogammarus) roeseli* GERVAIS and *Asellus aquaticus* (L.). Activity data are expressed by the amino acids μMol/mg albumen (60') = v_s), formed at 37.5 °C. $\bar{x}$ = arithmetic mean, n = number of investigations, s = standard error of mean

Species	Concentration of substrate	pH	$\bar{x}$	*n*	*s*
Gammarus	CGP $5 \cdot 10^{-3}$M	8.1—8.3	54.926	11	1.677
	CGT $5 \cdot 10^{-3}$M	7.0—7.1	44.481	4	3.601
Asellus	CGP 10^{-2}M	7.5—7.6	43.184	7	4.922
	CGT $6 \cdot 10^{-3}$M	7.1—7.2	33.602	7	1.171

Concerning the v_s values, significant differences can be found also between *Gammarus* and *Asellus* (for CGT : $P < 0.01$; for CGP: $0.02 > P > 0.01$). As related to Decapods some deviations in the stability of the enzyme can also be observed. In the case of *Gammarus*, an activity decrease of 20—25 per cent is perceiveable after 2 weeks for both CGT and CGP (at + 2 °C), and 35—40 per cent after 3 weeks in the case of *Asellus*. Even a storing of 5 weeks fails to evoke any considerable decrease in activity for the ferment of Decapods (cf. p. 45, ibid.).

In the gel filtration of the *Gammarus* extract we received consequently 3 fractions (peak) — against that of the Decapods (KLEINE, 1967) — of which the middle one was active for CGP and CGT, respectively *(Fig. 3).*

On the basis of the available data it may be inferred that one has to count with smaller or greater differences (e.g. deviating properties of the isoenzymes) in the proteolytic ferments present in the different groups of Crustacea (Decapoda, Amphipoda, Isopoda etc.) and even in the various species (C. MANWELL et al. 1967).

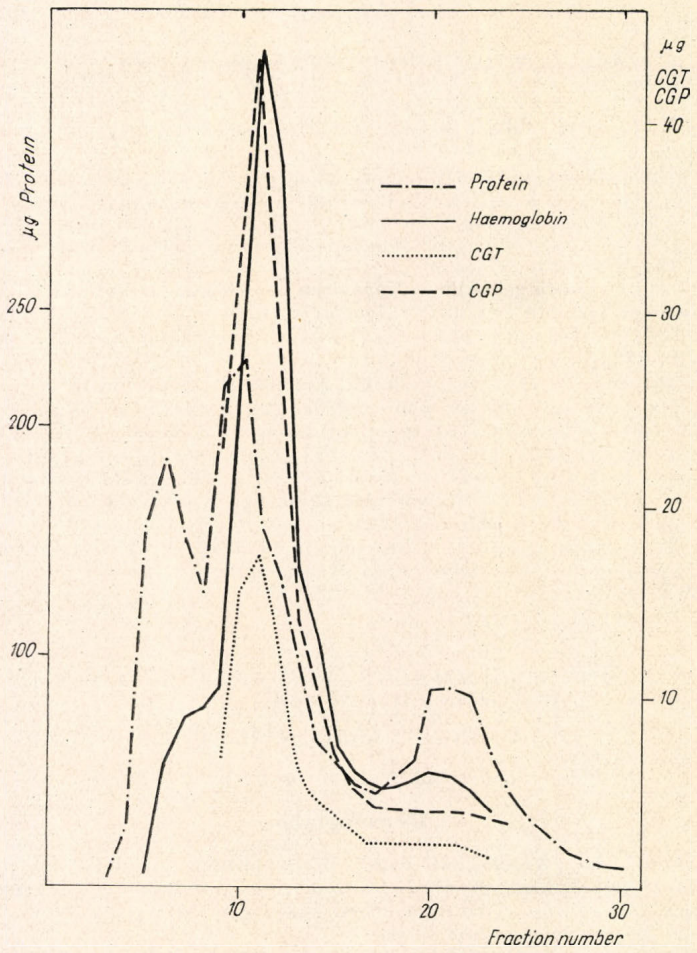

Fig. 3. The gel filtration on sephadex G-100 of the ferment extract of *Gammarus (Rivulogammarus) roeseli* GERVAIS. (For details see text)

Summary

1. The specific activity (v_s) of the hepatopancreas of *Gammarus (Rivulogammarus) roeseli* GERVAIS and *Asellus aquaticus* (L.) on carbobenzoxyglycyl-L-phenylalanine (CGP) and carbobenzoxy-L-glutamyl-L-tyrosine (CGT) is more than tenfold as that known for the other Decapod species.

2. The activity quotient of CGP/CGT (1.23 and 1.28, respectively) of the two investigated species refers, similarly to the case of *Astacus* and *Cambarus* (1.01 and 1.42, respectively), to a slight substrate specificity.

3. Of the 3 albumen fractions (peak) received during the sephadex gel filtration of the enzyme preparation of *Gammarus* only one was active with respect to CGP and CGT, respectively.

Acknowledgement

It is our agreeable duty to express our gratitude to Dr. R. KLEINE (Physiologisch-chemisches Institut, Halle /Saale(DDR) for his cordial help in our work.

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GAMMARUS (RIVULOGAMMARUS) ROESELI GERVAIS (AMPHIPODA) ÉS *ASELLUS AQUANTICUS* (L.) (ISOPODA) EXOPEPTIDÁZÁNAK NÉHÁNY TULAJDONSÁGÁRÓL

Ponyi Jenő, Biró Kálmán és *P.-Zánkai Nóra*

Összefoglalás

1. A *Gammarus (Rivulogammarus) roeseli* Gervais és *Asellus aquaticus* (L.) hepatopankreász carbobenzoxyglycyl-L-phenylalanin (CGP) és carbobenzoxy-L-glutamyl-L-tyrosin (CGT)-re vonatkoztatott specifikus aktivitás (v_s) több mint a tízszerese az eddig ismert Decapoda fajokhoz képest.

2. A két vizsgált faj CGP/CGT aktivitás quotiense (1,23 ill. 1,28) — az *Astacus* és *Cambarus*-hoz hasonlóan (1,01; 1,42) — csekély szubsztrát-specifikusságra utal.

3. A *Gammarus* enzimpreparátumának sephadex gélfiltrációja során kapott 3 fehérje frakció (peak) közül CGP ill. CGT-re vonatkozóan csak egy volt aktív.

НЕКОТОРЫЕ ХАРАКТЕРНИЕ СВОЙСТВА ЭКЗОПЕПТИДАЗ *GAMMARUS (RIVULOGAMMARUS) ROESELI* GERVAIS *(AMPHIPODA)* И *ASELLUS AQUATICUS* (L.) (ISOPODA)

Й. Поньи, К. Биро и *Н. П.-Занкаи*

1. Специфическая активность (v_s) гепатопанкреаса *Gammarus (Rivulogammarus) roeseli Gervais* и *Asellus aquaticus* L., расчитанная на карбобензоксиглицил-Л-фенилаланин (КГФ) и карбобензокси-Л-глутамин-Л-тирозин (КГТ) больше чем в 10 раз выше описанных до сих пор для Decapoda величин.

2. Соотношение КГФ/КГТ активности (1,23 и 1,28) этих видов также как у *Astacus* и *Cambarus* (1,01 и 1,42) указывает на незначительную специфичность субстрата.

3. В ходе очистки фермента *Gammarus* с помощью гельфильтрации было получено 3 белковых фракции, из которых в отношении КГТ только одна фракция обладала активностью.