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Purification and properties of soluble arylsulphatases isolated from hepatopancreas of the shrimp Crangon crangon L.

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Abstract

Arylsulphatase A and B were isolated from the hepatopancreas of the shrimp *Crangon crangon L.* after 7 days incubation in 50 ppm of detergent "SOLO". Arylsulphatase A, B–1 and B–2 were isolated from the hepatopancreas of the shrimp *C. crangon L.* incubated in pure brackish water (salinity 7‰). – Heavy and light fuel oil added to the enzyme in vitro in conc. of 2.0% inhibit the activity of arylsulphatase A in greater degree than arylsulphatase B (14.4% and 5.5% respectively). – Detergent "SOLO" (mixture of nonionic and anionic detergents) in the conc. of 0.5% inhibits for 58% arylsulphatase A and for 91% arylsulphatase B, whereas in conc. of 2.0% it inhibits arylsulphatase A for 91.7% and arylsulphatase B for 100%.

Zusammenfassung

Reinigung und Eigenschaften isolierter löslicher Arylsulphatasen aus dem Hepatopankreas der Garnele Crangon crangon L.

Arylsulphatase A und B wurden aus dem Hepatopancreas der Garnele *Crangon crangon* L. nach 7 Tagen Einwirkung von 50 ppm des Detergens "Solo" in Brackwasser (7‰ Salzgehalt) isoliert. Arylsulphatase A, B–1 und B–2 wurden aus dem Hepatopancreas von *C. crangon* aus sauberem Brackwasser isoliert. – 2% schweres und leichtes Heizöl hemmen in vitro die Enzymaktivität der Arylsulphatase A in stärkerem Maße als die Aktivität der Arylsulphatase B (14,4% bzw. 5,5% Hemmung). – Das Reinigungsmittel "Solo", eine Mischung aus nichtionischen und anionischen Detergentien, hemmt in der Konzentration von 0.5% die Arylsulphatase A zu 58%, die Arylsulphatase B zu 91%. Bei Einwirkung von 2,0% "Solo" wird Arylsulphatase A zu 91,7%, Arylsulphatase B zu 100% gehemmt.

Introduction

Arylsulphatase is an acid hydrolase of lysosomal origin hydrolysing esters of aromatic alcohols and sulphuric acid to an aromatic radical called "aryl", and hence the name of the enzyme.

There are three known arylsulphatases denominated arylsulphatase A, B and C. Arylsulphatases are widely spread in animals. Arylsulphatases A and B present in lysosomes, are well soluble in water and have great affinity to dipotassium salt

Scheme 1

Procedure of purification of soluble arylsulphatases of hepatopancreas of the shrimp *Crangon crangon* L.

STAGE I.

20% homogenate of hepatopancreas of *Crangon crangon* L. obtained by homogenization in 0.05 M TRIS-HCl Buffer pH 7.2 (TRITON X-100 concentration 0.05% added). Centrifuged for 20 min at 4000 x g.

RESIDUE I
(rejected)

SUPERNATANT I
Salted out to 20% with $/NH_4/_2SO_4$.
Mixture left for 4 hrs, later centrifuged for 15 min at 4000 x g.

STAGE II.

RESIDUE II
(rejected)

SUPERNATANT II
Salted out to 65% with $/NH_4/_2SO_4$.
Mixture left for 4 hrs, later centrifuged for 15 min at 8000 x g.

STAGE III

RESIDUE III
Dissolved in 0.01 M TRIS-HCl buffer, pH 7.2, later dialyzed in dest. water. After dialyzing to the residue, cooled to — 10°C acetone to concentration of 20% was added. After 2 hrs the suspension centrifuged for 15 min at 80 000 x g.

SUPERNATANT III
(rejected)

STAGE IV

RESIDUE IV
(rejected)

SUPERNATANT IV
Cooled acetone added to 65%. Centrifuged for 15 min at 80 000 x g.

STAGE V

RESIDUE V
Dissolved in 0.01 M TRIS-HCl buffer pH 7.2 and dialyzed in dest. water. Residue was applied to a DEAE-cellulose 1.5 × 12 cm column and eluted with increasing concentration of NaCl. Result of chromatography is shown on Figs. 1 and 2. Fractions of highest activity were condensed and dialyzed.

SUPERNATANT V
(rejected)

STAGE IV

The obtained fractions of arylsulphatases A, B–1 and B–2 were filtrated on a Sephadex G-200 column. The active enzyme was condensed to a volume of 5.0 ml.

of 2-hydroxy-5-nitrophenyl sulphate. Arylsulphatase C is present in microsomal fractions, almost insoluble in water with affinity to p-nitrophenyl sulphate (BARRETT, 1969).

Arylsulphytase A and B probably not only hydrolyse the sulphuric acid esters but play a role in the formation of the exoskeleton of Crustacea. Sulphuric phenol esters are involved in the process of hardening of chitin. High arylsulphatases activity during the formation of the exoskeleton in *Balanus eburneus* suggests an important role of these enzymes in this process (SHIMONY and NIGRELLI, 1972).

The role of sulphuric esters in animal tissues is of growing interest. Therefore we decided to investigate the properties of these enzymes in Crustacea. The first step in these investigations was the isolation and purification of arylsulphatases from the hepatopancreas of some crustacea.

Material and Methods

Arylsulphatases were isolated from the hepatopancreas of the shrimp *Crangon crangon L.* exposed 7 days to the 50 ppm of the detergent "SOLO" and from hepatopancreas of the control animals incubated in brackish water (7‰ of salinity) without detergent. The arylsulphatases were isolated by the method described in the paper of BŁESZYNSKI (1967). Arylsulphatase activity was determined by the method of ROY (1958), modified by BŁESZYNSKI (1967). The method of enzymes preparation is shown on the scheme 1.

Results and Discussion

The efficiency of arylsulphatase purification at each stage is presented in Table 1. The preparations from the control shrimps were 70-fold (arylsulphatase A), 99-fold (arylsulphatase B–1) and 107-fold (arylsulphatase B–2) purified. The enzyme preparations from the experimental shrimps were purified 70-fold (arylsulphatase A) and 63-fold (arylsulphatase B). Table 2.

Table 1

Purification of soluble arylsulphatases of hepatopancreas of the shrimp *Crangon crangon* L. (controls)

Stage of purification procedure	Protein content in whole fraction in mg	Total activity in μM NCS	Specific activity in μM NCS/mg protein	Degree of purification
STAGE I	238.0	47 792	201	1
STAGE III	91.0	126 284	1 392	7
STAGE IV	57.4	879 600	15 638	78
STAGE V				
Fraction A	12.1	221 556	18 463	92
Fraction B–1	15.0	264 825	17 655	88
Fraction B–2	11.7	268 971	22 989	114
STAGE VI				
Fraction A	6.3	126 949	20 151	100
Fraction B–1	8.1	161 741	19 968	99
Fraction B–2	16.4	352 551	21 497	107

Table 2

Purification of soluble arylsulphatases of hepatopancreas of the shrimp *Crangon crangon* L. after 7 days of exposure to 50 ppm of detergent "SOLO"

Stage of purification procedure	Protein content in whole fraction in mg	Total activity in μM NCS	Specific activity in μM NCS/mg protein	Degree of purification
STAGE I	386.0	138 000	350	1
STAGE III	85.5	172 790	2 010	6
STAGE IV	12.3	221 640	18 470	53
STAGE V				
Fraction A	11.0	198 660	19 600	53
Fraction B	10.7	221 929	20 741	59
STAGE VI				
Fraction A	8.0	196 624	24 578	70
Fraction B	9.5	210 263	22 133	63

This is a rather low degree of purification as compared with purification of these enzymes from other sources (GNIOT-SZULZYCKA, 1972; BIŁESZYŃSKI et al., 1969).

The yield of the purification was very high: the total enzymes activities were higher at the latter stages of purification than in the initial homogenates of the hepatopancreas, in the controls as well as in the experimental animals. It may be supposed that this phenomenon is caused by the presence of arylsulphatases inhibitors

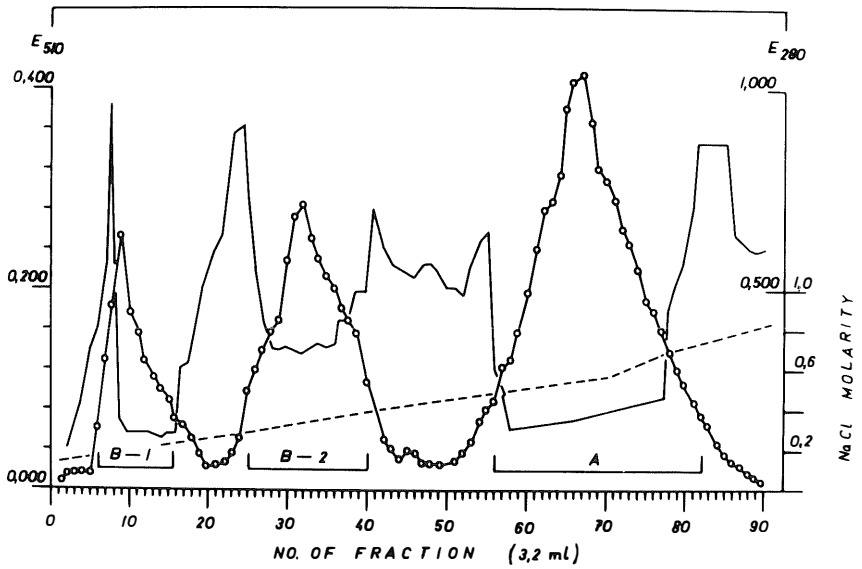

Figure 1

Chromatography of soluble arysulphatases of hepatopancreas of the shrimp *Crangon crangon* L. on a DEAE-cellulose column

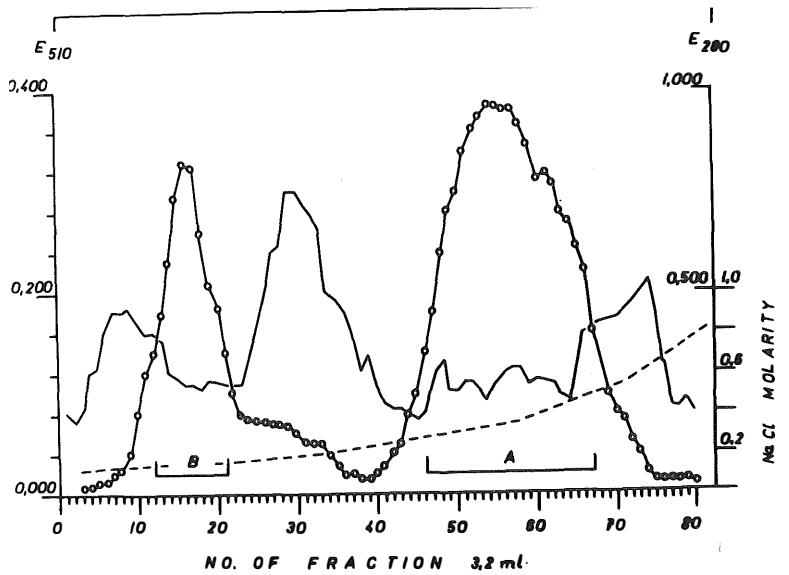

Figure 2:
Chromatography of soluble arysulphatases of hepatopancreas of the shrimp *Crangon crangon* L. on a DEAE-cellulose column, after 7 days of exposure to 50 ppm of detergent "SOLO"

——— activity of the enzyme
o-o-o-o protein concentration
— — — NaCl concentration

in the homogenates at the initial stage, followed by removing of these inhibitors later during the processes of purification of enzymes by means of column chromatography and dialysis.

From the hepatopancreas of the control shrimps three enzyme fractions were isolated: arylsulphatase A, B–1 and B–2, whereas from the homogenates of the hepatopancres of the shrimps incubated in the solutions of detergent "SOLO" only

Table 3
Effect of some pollutants on activity of soluble arysulphatases of the hepatopancreas of *Crangon crangon* L. (control)

Pollutant added	Final concentration of pollutants, percent	Percent of arylsulphatases activity in comparison to controls		
		A	B–1	B–2
Heavy fuel oil	0.5	97.6	100.0	98.1
	2.0	83.3	97.0	91.1
Light fuel oil	0.5	117.0	110.0	109.2
	2.0	97.0	86.3	89.0
Detergent "SOLO"	0.5	43.4	12.5	13.1
	2.0	10.8	0.0	0.0

two enzymes could be obtained: arylsulphatase A and B (Fig. 1, Fig. 2). It is supposed that this differences in the enzyme pattern may be caused by the toxic effect of detergent.

The effect of the detergent and of fuel oil on the activity of isolated arylsulphatases in vitro was investigated. It has been found that both pollutants inhibit the activity of the arylsulphatases in vitro, this effect being more pronounced in the case of detergent than in that of fuel oil (Table 3, Table 4).

Table 4

Effect of some pollutants on activity of soluble arylsulphatases of the hetatopancreas of the shrimp *Crangon crangon* L. after 7 days of exposure to 50 ppm of detergent ''SOLO''.

Pollutant added	Final concentration of pollutants, percent	Percent of arylsulphatases activity in comparison to controls	
		A	B
Heavy fuel oil	0.5	100.0	99.7
	2.0	85.6	94.5
Light fuel oil	0.5	106.4	104.2
	2.0	93.1	85.0
Detergent ''SOLO''	0.5	42.0	9.0
	2.0	8.3	0.0

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