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The arylsulphatase activity (E. C. 3.1.6.1.) in livers of different species of Antarctic animals *)

ABSTRACT: The arylsulphatase activity (E. C. 3. 1. 6. 1.) was compared in different species of Antarctic mammals, birds of the genus *Pygoscelis*, fish of the genus *Notothenia* and two crustaceans of the genus *Euphausia*. The role of arylsulphatase in the hydrolysis of sulphate phenol esters was pointed out. Considerable differences were observed in the arylsulphatase activity both within genera and species. But no differences in the activity of the enzyme examined were observed in relation to the sex or maturity stage of gonads of chosen Antarctic animals. The activity of arylsulphatase from liver and hepato-pancreas homogenates of Antarctic animals was lower than the activity of this enzyme in similar animals of the temperate zone.

Key words: Arylsulphatase, Antarctic crustaceans, fishes, birds and mammals

1. Introduction

Arylsulphatase is a lizosomal enzyme hydrolysing sulphate esters of aromatic hydrocarbons. In this reaction the bond between oxygen and sulphur is split.

On the basis of known properties of arylsulphatases from different species of animals and plants it has been determined that there are at least two basic types of this enzyme. They differ, amongst other things, by their affinity to substrates and kinds of inhibitors (Roy 1958).

Soluble lizosomal arylsulphatases A and B were distinguished and arysulphatase C belonging to the microsomal fraction. Lack of homogeneity was observed within arylsulphatases A and B and the enzyme was divided into fractions A-1, A-2 and B-1 and B-2, according to the order in which they eluted from the column with DEAE-cellulose (Błeszyński, Leżnicki and Lewosz 1969). Similar fractions were obtained from hepato-pancreas homogenates of shrimp *Crangon crangon* L. (Drewa et al. 1978).

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The best pH for arylsulphatase A and B is 5.0 and 6.0, respectively, and of substrate concentration 3 and 15 mM NCS. The method used for determining the arylsulphatase activity allows also to determine the total activity of both enzymes A and B at pH 5.6 and substrate concentration 12 mM NCS. Thus, further in the paper the term arylsulphatase covers the arylsulphatase activity A and B (Drewa et al. 1979 a, 1979 b).

Natural substrates of arylsulphatase A are sulphates of cerebrosides, of aspertic acid, and the natural substrate of arylsulphatase B is among others the sulphate of N-acetyloglucosamine (Błeszyński and Działoszyński 1965). Those natural substrates of both arylsulphatases are components of mucopolysaccharides from gelatinous film of animals and the chitin carapace of *Crustacea*. Because of the significance of this enzyme in the metabolism and physiology of animals its activity in the liver tissue abundant in hydrolases has been investigated and the activity of enzyme in liver of different animals of the Admiralty Bay (King George Island, South Shetland Islands) was compared. Two species of crustaceans were investigated: *Euphausia superba* Dana and *E. crystalloriophias* Holt et Tattersall, four species of fish: *Notothenia corriceps neglecta*, Nybelin, *N. rossi marmorata*, Fisher, *N. gibberifrons* Lönnberg, *N. nudifrons* Lönnberg and *Chaenocephalus aceratus* (Lönnberg), and also three species of birds: *Pygoscelis adeliae* (Hombron et Jacquinot), *P. papua* (Forster), *P. antarctica* (Forster) and three species of mammals: *Lobodon carcinophagus* (Hombron et Jacquinot), *Leptonychotes weddelli* (Lesson) and *Hydrurga leptonyx* (Blainville).

2. Material and methods

The arylsulphatase activity has been determined using the method described by Robinson (Robinson, Smith and William 1951) and modified by Błeszyński (Błeszyński, Leżnicki and Lewosz 1969). The substrate used — 2 hydroxy-5 nitrophenyl sulphate (NCS) — under the influence of the enzyme is hydrolysed to the remainder of sulphate and 4-nitrocatechol (4-NC) colorimetrically determined at 510 nm. The activity of the enzyme was determined in liver homogenates of vertebrates and in hepato-pancreas homogenates of invertebrates. Protein was determined after Lowry (Lowry et al. 1951). The activity of the enzyme was expressed in nM 4-NC per 1 mg of protein released during NCS hydrolysis in 10 min at 37 C. Also the best conditions for the hydrolysis of sulphate phenol esters with the participation of arylsulphatases were determined. The sex in *Euphausia* was identified on the basis of developed thelycum (females) or petasma (males) (Dzik and Jażdżewski 1978, Kittel and Presler 1980). The maturity stage of fish gonads was determined according to a 5-degree Maier's scale. Statistical significance was analysed using t-Student test.

3. Results and discussion

Both for mammals and Antarctic birds of a body temperature ca 39° C and for examined species of the genus *Euphausia* and fishes living in water

at a temperature −1 to +1° C the best temperature for hydrolysis of sulphate phenol esters by arylsulphatases is the temperature 37° C. Also the substrate concentration, pH and time of reaction answer the conditions of hydrolysis of homogenates of tissues of animals of the temperate zone.

Analyses of arylsulphatase activity in hepatopancreas of various development stages of *Euphausia superba* and in the liver of two species of fish *Notothenia corriceps neglecta* and *Chaenocephalus aceratus* differing as to gonad maturity do not show significant differences (Table I). And so the

Table I

Arylsulphatase activity in nM 4-NC (mg of protein/10 min.) 37° C in hepato-pancreas of krill and in livers of Antarctic fish examined

Taxon	Sex	Maturity stage	Arylsulphatase activity		Number of individuals examined
			$\bar{x}$	±S. D.	
	Juvenile forms		7.59	0.15	7
Euphausia superba		immature	7.82	0.17	7
	females	mature	7.62	—	2
		with eggs	7.73	0.14	9
	males	immature	7.63	0.15	5
		mature	7.66	0.12	14
		1	3.45	—	1
Notothenia corriceps	females	2	3.23	0.14	5
neglecta		3	3.43	0.21	6
		1	3.44	0.11	3
	males	2	3.36	0.27	5
		3	3.31	0.17	3
		2	4.80	—	2
Chaenocephalus	females	4	4.64	0.23	4
aceratus		5	4.75	0.14	4
	males	2	4.84	—	2
		3	4.82	—	—

examined activities of arylsulphatases from hepato-pancreas and liver are given only for females and males of particular species of crustaceans, fish, birds and mammals (Table II).

Among the Antarctic animals examined the mammals show the highest activity of arylsulphatase, it is slightly lower in crustaceans of the genus *Euphausia* (especially in *E. superba*), whereas the lowest activity is that of fish and among them in the species *Notothenia corriceps neglecta* (Table II).

The arylsulphatase activity in homogenates of tissues of animals from the temperate zone is higher than in that of animals from the Antarctic zone. For example, arylsulphatase activity in hepato-pancreases of shrimp *Crangon crangon* L. (*Crustacea*) is from 47 to 81 nM depending on the stage of the moulting cycle (Drewa et al. 1979 a). On the other hand the arylsulphatase of bream (*Abramis brama* L.) liver is 14.3 nM (Działoszyński, Kunik and Leżnicki 1966). Earlier investigations have shown also the higher arylsulphatase activity in livers of mammals of the temperate

Table II

Arylsulphatase activity in nM 4-NC/mg of protein/37° C/10 min. in hepato-pancreas and liver homogenates of some species of Antarctic animals

Taxon	Number of individuals examined	Arylsulphatase activity			
		Females		Males	
		$\bar{x}$	S.D.	$\bar{x}$	S.D.
Euphausia superba	42	7.63	0.15	7.70	0.12
Euphausia crystallorophias	28	10.08	0.20	10.05	0.07
Notothenia corriceps neglecta	22	3.37	0.17	3.36	0.35
Notothenia rossi marmorata	16	5.16	0.19	5.21	0.28
Notothenia gibberifrons	18	7.15	0.22	7.09	0.10
Notothenia nudifrons	16	9.86	0.12	10.17	0.10
Chaenocephalus aceratus	14	4.66	0.19	4.83	0.13
Pygoscelis adeliae	2	5.54	—	5.75	—
Pygoscelis papua	2	5.53	—	5.89	—
Pygoscelis antarctica	2	6.67	—	6.40	—
Lobodon carcinophagus	2	16.33	—	16.27	—
Leptonychotes weddelli	2	15.62	—	15.57	—
Hydrurga leptonyx	1	—	—	20.13	—

zone. For example, in the liver of *Mesocricetus auratus* Waterhouse, 80—100 nM of enzyme per mg of protein was recorded (Drewa and Zbytniewski 1974, Zbytniewski and Drewa 1973). Other tissues of mammals such as ox brain showed also a high activity of the enzyme (Błeszyński and Działoszyński 1965). This activity is several times higher as compared with the activity observed in the liver tissue of Antarctic animals.

It is difficult to interpret the observed differences in arylsulphatase activity in Antarctic animals examined. As it has been already mentioned the arylsulphatase catalyzes the hydrolysis of natural sulphur compounds such as sulphuric saccharides esters, steroids, bile pigments and aromatic phenols.

The cause of differences in the activity of the enzyme can be the kind of consumed and hydrolized food, the metabolic rate and isomorphous differences of the enzyme. Further detailed studies could explain the causes of these differences.

The differences in the arylsulphatase activity in the liver tissue of Antarctic animals examined are statistically significant ($p < 0.005$).

4. Summary

Statistically significant differences in the arylsulphatase activity were observed in liver homogenates of species examined of Antarctic animals (Table II). No significant differences were observed concerning the arylsulphatase content as regards the sex or development stage of gonads (Table I).

The best pH, time of reaction, temperature and concentration of substrate for the hydrolysis of sulphate phenol esters by the liver arylsulphatases in some Antarctic animals are as follows: 5.6; 10 min.; +37° C and 12 mM.

5. Резюме

Установлено сущесвенные статистические разницы активности арилсульфатазы в гомогенатах печени исследоваемых видов антарктических зверей (таблица II). Не констатировано сущестенных разниц содержания арилсульфатазы в пределах пола, а также в зависимости от стадии развития гонад (таблита I).

Оптимальное pH, аремя реакции, температура и сгущение субстрата для реакции гидролиза сульфатных эстров фенолов через арилсульфатазы печени у некоторых антарктических зверей являются: 5,6; 10 мин.; +37°C и 12 мМ.

6. Streszczenie

Stwierdzono istotne statystyczne różnice aktywności arylosulfatazy w homogenatach wątroby badanych gatunków zwierząt antarktycznych (tabela II). Nie stwierdzono natomiast istotnych różnic zawartości arylosulfatazy w obrębie płci oraz w zależności od stadium rozwoju gonad (tabela I).

Optymalne pH, czas przebiegu reakcji, temperatura i stężenie substratu dla przebiegu reakcji hydrolizy siarczanowych estrów fenoli przez arylosulfatazy wątroby u niektórych zwierząt antarktycznych są następujące: 5,6; 10 min.; +37° C i 12 mM.

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