POLISH POLAR RESEARCH (POL. POLAR RES.)	10	2	141—150	1989

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Proteolytic activity of Antarctic krill in relation to its feeding intensity in spring and summer

ABSTRACT: It was proved that the activity of basic proteinases (pH 8.3) and acid proteinases (pH 4.0) of the Antarctic krill increases exponentially in spring-summer season (September-December); the activity of the first ones is 6 times higher and increases more rapidly.

The positive relation between the proteolytic activity and the degree of gut filling of krill was also evidenced.

The lack of high activity of acid proteinases in early spring does not support the suggestions of Ikeda and Dixon (1982) that during Antarctic winter krill takes energy from the autoproteolysis of own body proteins.

Key words: Antarctic krill, proteolytic activity, intensity of feeding.

Introduction

Gaining knowledge on seasonal changes in the proteolytic activity of Antarctic krill is important from the food processing technological and ecological-physiological points of view.

Partial autoproteolysis was used in a practical method of obtaining from krill a chitin-free protein precipitate (Kołakowski et al. 1984). Autoproteolysis is here a factor facilitating the degradation of muscle flesh proteins in a solution of low ionis strength (Kołakowski et al. 1980, Kołakowski and Lachowicz 1982) and also a factor diminishing the viscosity of water-krill homogenates (Kołakowski 1983). Yearly cycle of changes in the proteolytic activity of krill at pH 7.5, which were described in the previous paper (Kołakowski 1986) indicate a need for adjusting the technological parameters of krill processing to the particular fishing season.

The physiology of Antarctic krill is still insufficiently known. In few papers on this subject a very high metabolism rate in Antarctic krill (Rakusa-Suszczewski and Opaliński 1978, Ikeda and Mitchell 1982) and the role of lipids as the basic source of energy (Clarke 1980, 1985; Kołakowska 1985) were stressed. From studies on other crustaceans it follows that also carbohydrates, particularly those occurring in the unbound state, constitute an easily accessible source of energy (Partvathy 1971). In contrast, the role of proteins as a source of energy in Antarctic krill is controversial. In the literature, no information on this subject have been found, although some researchers (Ikeda and Dixon 1982, Ikeda and Bruce 1986) suggested that the survival of krill in the winter season is related to the utilization of body proteins for energetic purposes.

If the above statement is correct then in Antarctic winter there should occur an increase in the activity of intercellular proteases in starving krill, and a decrease in this activity after the resumption of normal feeding. It is commonly assumed that the activity of proteases, particularly those of the cell organella, is proportional to the use of cellular proteins for energetic requirements (Love 1970, Barrett 1977a, Sauer and Haider 1979, Konagaya 1982).

The aim of the present study was to investigate the proteolytic activity of Antarctic krill in the spring-summer season (September-December), in which most significant changes in krill feeding activity occur. Assuming that the proteolytic activity investigated at pH 8.3 is related mainly to digestive enzymes while the proteolytic activity at pH 4.0 to acid proteinases (Kimoto, Thanh and Murakami 1981, Nishimura et al. 1983, Kawamura et al. 1984, Osnes and Mohr 1985a, b) an attempt was made to determine the interdependence between krill proteolytic activity and its feeding intensity.

Material and methods

Antarctic krill (*Euphausia superba* Dana) was caught in the Admiralty Bay (King George Island, South Shetlands) over the period 30 September — 12 December 1986, using a pelagic trawl with a 2 m × 1 m rectangular mouth, towed by the motor boat "Dziunia".

Alive captured animals were brought to the laboratory of the "H. Arctowski" Polish Polar Station and immediately analysed.

The degree of alimentary tract filling was determined using a 5-grade scale (Rakusa-Suszczewski 1982).

A sample of 100—150 g (about 200—300 specimens), depending on the amount of krill captured in a given catch (0.5 to 5 kg), was homogenized in a fast rotating mixer (2—5 s) to obtain a homogenous mass.

20 g of such homogenate and 100 cm^3 of distilled water cooled to a temperature of about 2°C were put into the jar of an MPW-309 universal laboratory aid (Mechanika Precyzyjna, Warszawa) and homogenized twice for 30 s (at the interval of 1 min.) at 10000 rpm, and then transfered into test-tubes and centrifuged for 10 min. at 35000 × g. The supernatant obtained was filtered and considered a "raw" enzyme.

Proteolytic enzyme activities were estimated using as a substrate a hemoglobin denatured with urea (Mejbaum-Katzenellenbogen 1968), at a proper pH value (8.3 or 4.0). 5 cm^3 of the enzyme was added to the proper sample consisting of 20 cm^3 of buffer and 15 cm^3 of the substrate heated to 37°C and the total was then incubated in a thermostate with a magnetic mixer (ZW2/27 Type Sp. Horyzont, Kraków) for 20 min., at temperature controlled to the nearest 0.1°C. The autolysis was stopped by adding 250 cm^3 of 5% TCA solution, and then the whole content was homogenized, left for 30 min and then filtered. Trichloroacetic acid was added to the control sample before starting the incubation. Following buffers were used: 0.2 M borate buffer of pH 8.3 and 0.2 M acetate buffer of pH 4.0. Protein hydrolyse products (PHP) in TCA-extracts were determined according to Lowry et al. (1951) using bovine serum albumine as a standard; protein in the crude enzyme krill extract was determined by biuret method (Gornall, Bardawill and David 1949). Autoproteolytic krill activity was measured similarly as in the former paper (Kołakowski 1986) by incubating krill homogenate with phosphate buffer (I = 0.05; pH = 7.5) at 40°C for periods of 0, 5, 10, 15 and 20 min.

Parameters of proteolyse speed were calculated from a first-order reaction kinetic equation.

Results

Figure 1 presents the changes of the whole krill proteinase activities against denaturated hemoglobin at pH 8.3 and 4.0 during spring-summer season. It was proved that irrespectively of pH values of the substrate krill proteolytic activity increased in time according to the exponential curve, the time measured in subsequent days to the exponential curve, the time measured in subsequent days of the calendar year. In the period from 29 September to 12 December 1986 (79 days) the proteolytic activity increased about 8 times in alkaline environment (pH 8.3) and about 6 times in acidic environment (pH 4.0).

Proteolytic activity in alkaline environment was 5.2 times higher than the activity in acidic environment at the beginning of the study period and over 6.9 times higher at its end. This evidences that in the investigated

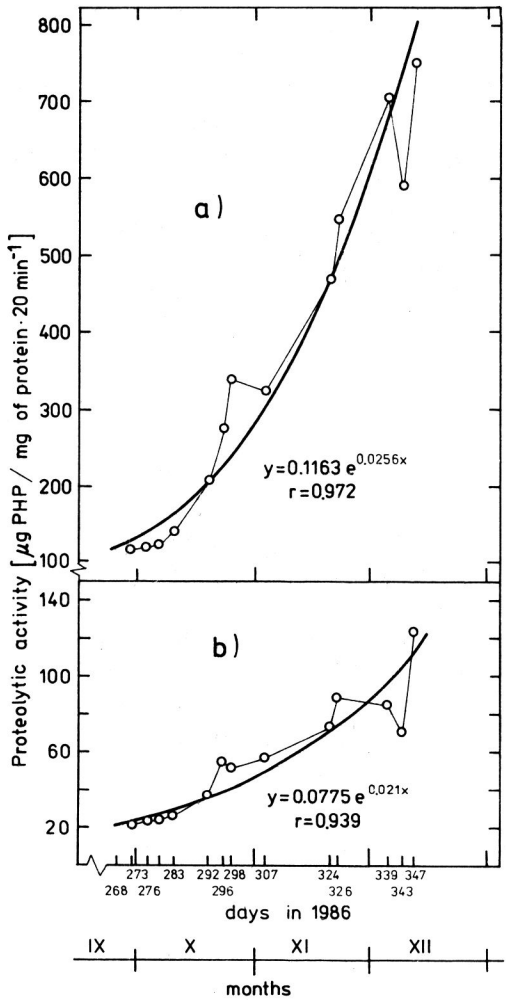

Fig. 1. Changes in proteinase activities of whole krill extracts against hemoglobin at pH 8.3 (a) and pH 4.0 (b) during spring-summer season

period an increase in proteolytic activity in alkaline environment occurred slightly quicker than in acidic environment.

Figure 2 shows the interrelation between the proteolytic activity and the degree of krill alimentary tract filling. These investigations were based on the material captured in the period of the fastest growth and feeding activity of krill, i.e. between 6 October and 22 November 1986. At the beginning of this period krill catches were dominated by non-feeding specimens of pinky-coloured cephalothorax, whereas at its end the stomachs and hepato-pancreas of all captured animals were filled with food; the characteristic green spot was clearly visible. A highly significant statistical correlation

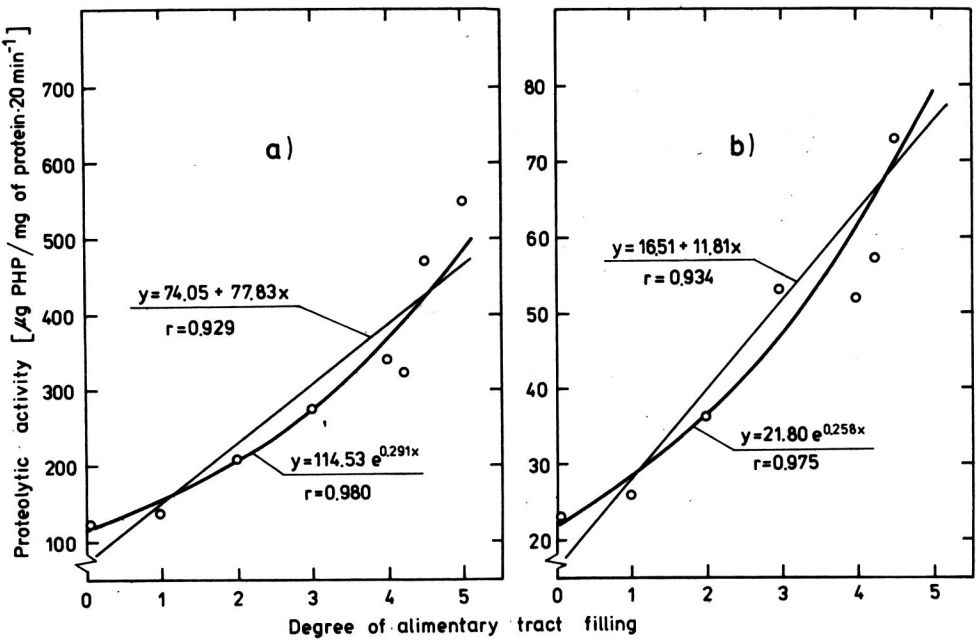

Fig. 2. Interrelation between the degree of alimentary tract filling and proteinase activities of whole krill extracts against hemoglobin at pH 8.3 (a) and pH 4.0 (b) during spring--summer season

($\alpha < 0.001$) has been observed between the degree of krill alimentary tract filling and its proteolytic activity, both at pH 8.3 and pH 4.0 (Fig. 2).

From the calculated linear relation it follows that an increase in the degree of alimentary tract filling by 1° corresponds to the increase in the protein hydrolysis products (PHP) amounting on average to 77.83 g/mg of total raw enzyme protein in alkaline and 11.81 g/mg in acidic environment after 20 min of incubating a sample at the temperature of 37°C. This means that in the investigated period an increase in the proteolytic activity at pH 8.3 was more than 6.5 times faster than that at pH 4.0.

A more detailed mathematical analysis of the results obtained reveals that the relation between the increase in krill proteolytic activity and the degree of alimentary tract filling is not a linear but an exponential one (Fig. 2).

In table 1 the results of investigations concerning the influence of starved krill on their autoproteolytic activity at pH 7.5 are presented. These investigations were carried out on krill captured on 25 October 1986 of various size and with stomachs and hepatopancreas maximally filled with green food. A sample of the fed krill (F) was taken immediately after its transporting to the laboratory. A sample of the starved krill (S) was taken for analysis after keeping it for 3 days in filtrated sea water.

Table 1

Comparison of the parameters of autoproteolytic activity (pH 7.5) in fed (F) and starved (S) krill

Parameters	Symbols	4%TCA-soluble products of protein hydrolysis (PPH)				4% TCA-soluble peptides			
		recalculated for krill dry weight		recalculated for krill total protein (N × 6,25)		recalculated for krill dry weight		recalculated krill total pro (N × 6,25)	
		F	S	F	S	F	S	F	
Reaction rate constant	$\bar{K}$	0,10354	0,09867	0,10346	0,09868	0,15750	0,12025	0,15750	0,
Initial reaction rate (g 100 g min^{-1})	V_0	0,394	0,216	0,615	0,305	0,330	0,201	0,516	0,
Mean reaction rate (g 100 g min^{-1})	X_x	0,255	0,122	0,398	0,172	0,190	0,104	0,297	0,
Reaction half-time	$t^1/_2$	6,69	7,02	6,69	7,02	4,40	5,76	4,40	5,

This krill was in condition, and specimens with a green spot in their cephalothorax constituted only 3 % of the sample; the degree of alimentary tract filling of the remaining individuals was about 1.5.

The starving of krill caused the about two fold decrease in the intial (V_0) and mean ($V_{\bar{x}}$) reaction rate, independently of the kind of investigated products of protein hydrolysis (total hydrolysis products (PHB), peptides) and of the way of calculating the results (for dry weight, for total protein). The value of the reaction rate constant ($\bar{K}$) decreased by about 5 % for PHB and about 23 % for peptides, while the reaction half life time increased by about 5 % and about 30 %, respectively (Tab. 1). These data evidence for the decrease in krill autoproteolytic activity together with the decrease in their alimentary tract filling.

Discussion

Our results allow to suggest that in winter krill exhibits a low proteolytic activity both with respect to digestive enzymes and to the enzymes of the muscular tissue. Together with the beginning of the feeding period, which takes place in the Antarctic at the beginning of October, krill proteolytic activity clearly increases and the linear increase in the alimentary tract filling is accompanied by an exponential increase in proteolytic activity.

These results are fully concordant with the previously determined sinusoidal changes in krill proteolytic activity over the year (Kołakowski 1986). It has to be stressed that the activity of enzymes isolated from krill (alive specimens were taken for investigations) was investigated against the denatured hemoglobin, which is considered the most suitable exogenous protein substrate for the proteases of Crustacea and Asteroidea Kozlovskaya and Vaskovsky 1970) and that the measurements of the proteolytic activity was conducted simultaneously at pH 8.3 and pH 4.0.

This enables the activity of two different enzyme groups to be determined; namely it is well known that krill enzymes acting in a slightly alkaline environment correspond mainly to digestive enzymes, which are relatively resistant to high pH values, but become quickly inactivated in acidic environment (Osnes and Mohr 1985b). In contrast, the activity determined at pH 4.0 corresponds mainly to cathepsins, which play a vital role in the physiological protein degradation (Barrett 1977a, b). The occurrence of cathepsins A, B, H and L in Antarctic krill was proved by Nishimura et al. (1983) and Kawamura et al. (1984).

The lack of high activity of acidic proteinases in the early spring period in Antarctic krill may testify against the assumption of Ikeda and Dixon (1982) according to which krill obtains energy from the proteolytic degradation of their own body proteins in winter. It seems more probable

that in the winter period krill extracts energy from simpler and more easily available forms of nitrogen, such as free aminoacids, peptides and others, because the contents of non-protein nitrogen strongly fluctuates in krill seasonally, while the contents of proper proteins changes insignificantly (Kołakowski, unpubl.).

The much higher (about 6 times) proteolytic activity at pH 8.3 in comparison with this activity at pH 4.0 evidences to the fact that Antarctic krill has very active digestive enzymes, well adapted to low water temperature in which the animals live, as well as to the comparatively short period in which food occurs in abundance. Consequently in krill autoproteolysis the main role is played by the enzymes acting at slightly alkaline or neutral pH. The activity of these enzymes increases very quickly together with krill feeding intensity increase. The presence of food, namely of phytoplankton, is the stimulus causing the secretion of these enzymes to the alimentary tract. Hence, it might be assumed that the occurrence of the green spot in the cephalothorax is a practical indicator of high krill proteolytic activity. A still better indicator of proteolytic activity is the degree of filling of alimentary tract. Accordingly, it seems that krill proteolytic activity may be a good indicator of krill metabolic activity, especially its anabolism, and also an indirect indicator of body weight increase of this crustacean.

The studies were performed at the "H. Arctowski" Station during Xth Antarctic Expedition of Polish Academy of Sciences as a part of CPBP 03.03. Project.

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Received May 15, 1988
Revised and accepted February 2, 1989

Streszczenie

Wykazano, że aktywność proteinaz zasadowych (pH 8.3) i kwaśnych (pH 4.0) kryla antarktycznego rośnie wykładniczo w okresie wiosenno-letnim (wrzesień-grudzień) z tym, że aktywność tych pierwszych jest około 6 razy wyższa i wykazuje szybszy przyrost niż tych drugich.

Stwierdzono wyraźną, dodatnią zależność pomiędzy aktywnością proteolityczną a stopniem wypełnienia przewodu pokarmowego kryla. Brak wysokiej aktywności proteinaz kwasowych w okresie wczesno-wiosennym nie potwierdza przypuszczeń Ikedy i Dixona (1982) jakoby w okresie antarktycznej zimy kryl czerpał energię z autoproteolitycznego rozkładu białka własnego ciała.