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Seasonal variation of autoproteolytic activity in the Antarctic krill, *Euphausia superba* Dana *

ABSTRACT: Seasonal changes in the Antarctic krill (*Euphausia superba* Dana) autoproteolytic activity were followed throughout the year. Using the kinetic formula for the first order reaction, the initial reaction rate (y_0), the rate after 5 minutes (y_5) and the average reaction rate ($y_{\bar{x}}$) after 0, 5, 10, 15 and 20 min of incubation of mixed homogenate at $40° \pm 0.2°C$ were determined in each sample. Changes in the krill autoproteolytic activity over the year were found to follow a sinusoid with a maximum during the austral summer (January) and a minimum during the austral winter (July-August). The maximum initial reaction rate was about ten times the minimum initial rate, which is an evidence of a considerable seasonal variation in the krill autoproteolytic activity associated presumably with the krill feeding intensity.

Key words: Antarctic, krill, autoproteolytic activity, seasonal changes.

1. Introduction

The Antarctic krill, *Euphausia superba* Dana, contains very active proteases (Noguchi et al. 1976, Seki et al. 1977, Chen et al. 1979, Jakubiec-Puka et al. 1983, Kimoto et al. 1981, 1983, Fik 1984), which makes the animals susceptible to autolysis resulting in protein degradation to polypeptides, peptides and amino acids (Kołakowski and Lachowicz 1982). Autoproteolysis affects both the technological utility of krill as a raw material for processing and its stability on storage. Moreover, a possibility cannot be excluded that the proteolysis

intensity is related to the krill feeding intensity and can be a measure of the animal's physiological activity, growth rate included.

The krill autolysis dynamics and effects of pH, temperature, incubation time and other factors are known fairly well (Kuwano et al. 1976, Doi et al. 1978, Kubota and Sakai 1978, Kanagaya 1980, Kołakowski et al. 1980a, 1980b, Christians and Leinemann 1983), although some of the studies listed were based on assays made on frozen krill, stored for a long time. On the other hand, data on seasonal changes in the krill proteolytic activity are missing altogether.

The aim of the present work was to follow, throughout a year; seasonal variations in the krill proteolytic activity during the autolysis carried out under optimal conditions; an attempt to describe the changes mathematically was made.

2. Material and methods

The Antarctic krill was being caught over the period of 28 Dec.1983—
—27 Nov.1984 in the Admiralty Bay (the main sampling site coordinates: 61°08 S latitude; 58°26 W longitude) by means of a pelagic trawl a 2 m × 1 m rectangular mouth, towed by the motor boat "Dziunia".

Immediately after capture, the live krill catch was brought to the laboratory and, after a short (5—15 min) period in aerated sea-water, sampled for the assays. Depending on the catch magnitude (usually 0.5—5 kg), a 100—200 g sample (about 200—400 krill individuals) was taken and homogenized for 5—10 s in a fast rotating mixer to a homogenous mass.

A 10 g homogenate sample was transferred to a jar and supplied with 50 cm³ phosphate buffer (15.5 mM Na_2HPO_4 + 3.38 mM KH_2PO_4 per 1000 cm³) of pH 7.5, preheated to 40°C and incubated in a 5-site ZW-2/27 type thermostatic unit (Sp. "Horyzont", Kraków) at 40° ± 0.2°C at the maximum speed of magnetic mixer (250 rpm). The optimum temperature of krill autoproteolysis when mixing with water had been determined previously (Kołakowski et al. 1984). The incubation time was 0, 5, 10, 15 and 20 minutes. The autolysis was stopped when necessary by adding 250 cm³ of 5% TCA to the jar and homogenizing its contents 3 times for 60 s at 5-min intervals at 13000 rpm, in an MPW-309 universal laboratory aid (Mechanika Precyzyjna, Warszawa). Subsequently, the homogenate was filtered through hard paper filters into a conical flask.

The amount of 4% TCA-soluble products of protein hydrolysis (PPH) was determined according to Lowry et al. (1951) with a crystallised bovine albumin (BDH Chemicals Ltd) as standard. The samples, treated

with the Folin-Ciocialteau reagent, were allowed to stand for 3 h (reaction time). Absorbance was measured in a Unicam SP6–500 spectrophotometer at 750 nm, 1 cm path-length.

The autoproteolysis parameters were determined from the first order reaction kinetic equation:

$$K_1 = \frac{1}{t_i} \ln \frac{x_{max} - x_0}{x_{max} - x_i}$$

where:

x_{max} = the amount of TCA-soluble PPH (protein hydrolysis products) in a sample after the maximum reaction time (20 min), in mg per g pure protein,

x_0 = the initial amount of TCA-soluble PPH at zero reaction time, in mg per g pure protein,

x_i = the amount of TCA-soluble PPH after the incubation time i (= 5, 10 or 15 min), in mg per g pure protein,

t_i = incubation time (5, 10 or 15 min).

The value of K_0 was calculated by regressing t_i on K_i, with K_0 being the regression equation coefficient a. The initial reaction rate (V_0) was calculated from the formula:

$$V_0 = K_0 (x_{max} - x_0)$$

The reaction rate after 5 min ($V_{5'}$) and the average reaction rate ($V_{\bar{x}}$) are:

$$V_{5'} = K_5 (x_{max} - x_5) \quad \text{and} \quad V_{\bar{x}} = \frac{V_0 + V_5 + V_{10} + V_{15}}{4}$$

respectively; all V values being in mg TCA-soluble PPH per g pure protein per min.

Pure protein in krill homogenates was determined by the Kjeldahl method from the difference between total nitrogen and non-protein TCA--soluble nitrogen, the conversion factor being 6.25.

3. Results

Generally speaking, annual changes in the krill autoproteolytic activity follow a cycle with a clear-cut maximum in the austral summer and a minimum in winter.

A mathematical description of the changes is fairly difficult owing to their varying intensity in different seasons, and also owing to the lack of winter data (June, July), when the boat was out of order.

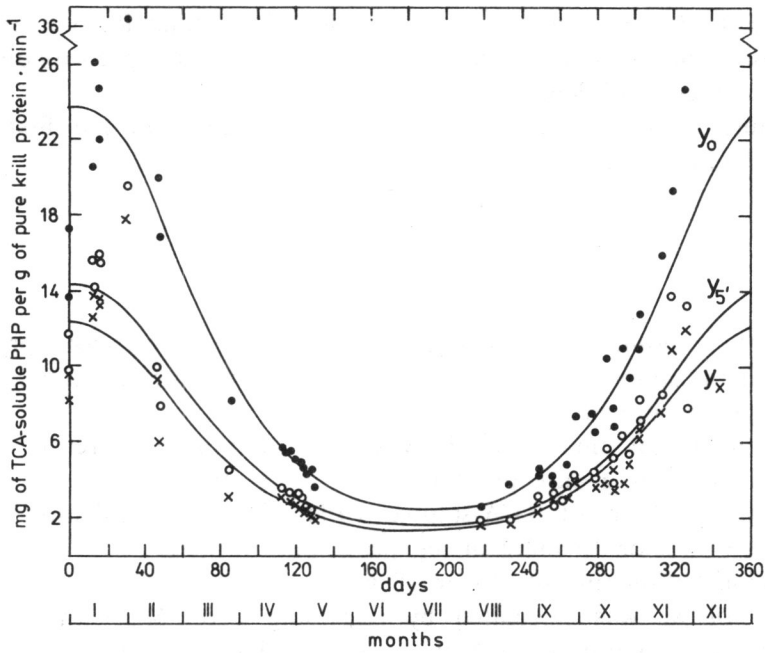Mathematical analysis of the results obtained showed the sinusoidal

Fig. 1. Relationship between the first order reaction rate of krill autoproteolysis and the consecutive day of the year, as described by the sinusoidal equation:

$$y = \exp \{a + b \cdot \sin [\omega/x - 1/\varphi]\}$$

where:

y = reaction rate (mg TCA-soluble PPH per 1 g pure krill protein per minute) after 0 (y_0), 5 minutes (y_5), and the mean ($y_{\bar{x}}$) after 0, 5, 10, 15 and 20 minutes incubation of the sample at $40° \pm 0.2$ C, a = harmonic mean of the results over the period of study (365.25 days), b = maximum amplitude, $\omega = \dfrac{2}{365.25}$, x = number of the day in the calendar year, φ = phase shift

equation to provide the most adequate description of seasonal changes (Fig. 1). The equation parameters, calculated from the original data, are presented in Table I. The extremes of the function given allow to conclude on the maximum and minimum reaction rates and on the day of the

Table I

Parameters of sinusoidal equation: $y = \exp \{a + b \cdot \sin [\omega/x - 1/\varphi]\}$

Reaction rate	Values of coefficients			Extremes of the function			
	a	b	φ	x_{max}	x_{min}	y_{max}	y_{min}
y_0	2.0158	1.1555	1.4758	6.62	189.15	23.84	2.36
y_5	1.5278	1.1327	1.5012	5.05	187.67	14.30	1.49
$y_{\bar{x}}$	1.3780	1.1300	1.5363	3.01	185.63	12.28	1.28

year they will occur. The maximum activity for the initial reaction rate (y_0) will occur on day 7 (6.62 to be exact) of the year; the minimum will occur on day 189; the representative values are 23.84 and 2.36 mg TCA-soluble protein hydrolysis products per g pure protein per minute (Table I). One can assume then that the extremal value, as determined by the sinusoidal equation, of the initial reaction rate in the Antarctic summer is about 10 times the winter value. Thus an exceptionally extensive seasonal variation in the krill autoproteolytic activity is documented.

4. Discussion

The assays reported in this paper were made on whole live krill individuals subject to a short and rapid homogenation prior to the enzymatic analysis. In this situation, the main role in the autoproteolysis was played by the visceral enzymes of the cephalothorax which, as show by Kanagaya (1980), are responsible for most of the proteolytic activity in krill body. This is also confirmed when comparing results of the autoproteolytic activity studies carried out separately in the summer krill cephalothorax and abdomen homogenates; those results show the initial reaction rate (y_0) to be higher by about 20 times in the first case than in the other (Kołakowski, unpubl. data). It is then suggested than the extensive seasonal variation in the krill proteolytic activity, found in the present work, is mostly associated with the krill feeding activity. The highest activity was typical of the "green" krill, frequently found in summer catches. Conversely, the krill harvested in winter, with empty alimentary tract indicating the lack of feeding, showed the lowest proteolytic activity. This is an evidence of the fact that during krill feeding and starving, exogenous and endogenous conditions alter, which affects the activity of proteinases. The factors that play a role in regulating proteinase activities in animal tissue, e.g., mechanical activity, hormone levels, redox state, energy level, proteinase inhibitor content, nutritional changes, changes in molecular or some other properties of substrate proteins are extensively treated in the literature (Barrelt 1977, Elödi 1984). It is difficult to pinpoint those most important for krill proteinases. Since the feeding intensity plays the fundamental role in seasonal changes of the krill autoproteolytic activity, it seemed reasonable to think that the rate of autoproteolysis was controlled mainly by the rate of digestive enzymes secretion and their transfer to a fluid of the digestive tract.

The cycle of seasonal changes in the krill autoproteolytic activity coincides markedly with the annual cycle of Antarctic phytoplankton primary production (El-Sayed 1968, Everson 1977, Ligowski 1986), the latter — as shown by Astheimer et al. (1985) — directly affecting the

krill growth rate. Since proteins, as a material for tissue build-up, cannot be substituted by other nutritional elements and have to be provided from outside, that is from food, one can assume with a high probability that the autoproteolytic activity is a reliable indicator of krill feeding intensity and, indirectly, of krill growth rate. It has to be, however, confirmed by additional studies dealt with in the next paper.

5. Резюме

Исследовались сезонные изменения автопротеолитической активности антарктического криля в годовом цикле. В каждой пробе оценивались скорость первоначальной реакции (y_0), скорость реакции через 5 минут (y_5), а также средняя скорость реакции после 0, 5, 10, 15 и 20 минутах инкубации гомогената при 40°C ($y_{\bar{x}}$), используя кинетические уравнение для реакции 1 ряда. Изменения автопротеолитической активности криля в течение года характеризуется синусоидой (рис. 1) с максимальным значением в течение антарктического лета (январь) и минимальным — в течение антарктической зимы (июль-август). Параметры уравнения этой кривой представлены в таблице I. Максимальные значения скорости первоначальной реакции летом являются десятикратно большими, чем минимальные значения, зарегистрированные зимой. Это указывает на большую сезонную изменчивость автопротеолитической активности, что связано, вероятно, с изменяемой интенсивьостью питания криля.

6. Streszczenie

Prześledzono sezonowe zmiany autoproteolitycznej aktywności kryla antarktycznego w cyklu rocznym. W każdej próbie oceniono tempo reakcji początkowej (y_0), tempo reakcji po 5 minutach (y_5) oraz średnie tempo reakcji ($y_{\bar{x}}$) po 0, 5, 10, 15 i 20 minutach inkubacji homogenatu w 40°C stosując równanie kinetyczne dla reakcji pierwszego rzędu. Zmiany autoproteolitycznej aktywności kryla w ciągu roku obrazuje przebieg sinusoidalnej krzywej (Fig. 1) z maksimum w ciągu antarktycznego lata (styczeń) i minimum w ciągu antarktycznej zimy (lipiec-sierpień). Parametry równania tej krzywej zestawione są w tabeli I. Wartości maksymalne tempa reakcji początkowej w lecie są około 10-krotnie wyższe, niż wartości minimalne rejestrowane zimą. Wskazuje to na dużą sezonową zmienność autoproteolitycznej aktywności, co jest prawdopodobnie związane ze zmienną intensywnością odżywiania się kryla.

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