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Tissue-specific distribution of pyruvate kinase isoforms improve the physiological plasticity of Northern krill, *Meganyctiphanes norvegica*

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Abstract

The Northern krill, *Meganyctiphanes norvegica* (Crustacea, Euphausiacea) is widely distributed in the northern and northeastern parts of the Atlantic Ocean where it faces rapid variations in water temperatures and food. We studied the physiological potential of krill to compensate for environmentally induced metabolic changes. Two isoforms of the glycolytic key enzyme pyruvate kinase (PKI and PKII, EC 2.7.1.40) were partly purified from *M. norvegica* by anion exchange chromatography. Specific activities and catalytic properties of each isoform were determined in whole body extracts as well as in selected organs and tissues of males and females. Both PK-isoenzymes differed slightly in their temperature profiles, their activation energy and their molecular weights. PKI showed a high affinity for the substrate PEP and was not affected by fructose-1.6-bisphosphate (FBP). In contrast, PKII showed low affinity for PEP but was strongly activated by FBP, up to 40-fold. The specific PK-activity of whole organisms was lower in females (44.9 ± 4.8 U $\cdot$ g_{ww}^{-1}) than in males (61.3 ± 7.7 U $\cdot$ g_{ww}^{-1}). In females PK II represented 20% of the total PK-activity while it was only 10% in males. Highest PK activities were present in the hearts, the eyes, pleopods and in the thoracopods. In the stomachs and the midgut glands PK activities were low. Almost all organs contained PKI and PKII. However, PKI prevailed in the abdomens, the pleopods, the thoracopods, and in the thoracic muscles. PKII dominated in the eyes, the midgut glands and in the ovaries. Experiments showed that the tissue concentrations of FBP increased with food uptake and temperature. The expression of two PK-isoforms with different kinetic properties and the mediation of substrate affinity by FPB is a powerful tool to immediately regulate glycolytic energy flows in different organs. The krill is capable of adjusting energy consumption to changes in nutritional conditions as well as variations of environmental temperatures.

Keywords: Fructose-1.6-bisphosphate; Isoenzymes; *Meganyctiphanes norvegica*; Nucelotides; Pyruvate kinase

1. Introduction

The Northern krill, *Meganyctiphanes norvegica* (M. Sars) (Crustacea: Euphausiacea) is an abundant planktonic crustacean in the northeast Atlantic and adjacent seas (Mauchline, 1960; Mauchline and Fischer, 1969). It performs extended diurnal vertical migrations and has to spend substantial metabolic energy to remain pelagic (Kils, 1981; Tarling et al., 1998). Northern krill live at water temperatures from 2 to 16 °C and appear in high productive as well as in oligotrophic waters (Einarsson, 1945; Lindley, 1982). This way of life demands a high physiological flexibility towards var-

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iations of environmental temperatures as well as trophic conditions. Accordingly, a high potential of metabolic regulation must be expected in terms of energy flow, substrate turnover, and, thus, the performance of metabolic key enzymes.

We focused our interest on pyruvate kinase (PK, EC 2.7.1.40), a glycolytic key enzyme with high regulatory potential (Michaelidis and Storey, 1990; Oeschger and Storey, 1990; Bonamusa et al., 1992; Lazou and Frosinis, 1994). In mammals, at least three different isoenzymes of PK were identified. The type M isoenzyme appears in the skeletal muscle, the heart and the brain. It shows hyperbolic kinetic properties enabling high glycolytic turnover rates. Type L is present in tissues such as liver and kidney which carry out gluconeogenesis. This isoform shows cooperative substrate binding properties and is controlled by hormones. The third isoenzyme, type A, is located in most other tissues with intermediate glycolytic properties and is the predominant form during early development and in neoplastic tissues (Imamura and Tanaka, 1972; Imamura et al., 1972).

In crustaceans two distinct isoenzymes (PKI and PKII) were isolated. However, their tissue distribution and their detailed physiological roles are not sufficiently investigated yet (Lesicki, 1976; Guderley and Hochachka, 1977). Previous work on Northern krill showed that the activity of either isoenzyme was regulated differently. PKI did not respond to fructose-1.6-bisphosphate (FBP) while PKII was directly affected by FBP (Salomon et al., 2000).

In order to study the roles of the pyruvate kinase isoenzymes in the regulation of metabolic performance in the Northern krill, *M. norvegica*, we investigated the properties, the distribution and the specific activities of both isoenzymes in different organs and tissues. Additionally we maintained krill in the laboratory at different temperatures with food and without food and measured the tissue concentrations of ATP and FBP, which are the major effectors of PK.

2. Materials and methods

2.1. Origin of animals

M. norvegica were captured with a multi-net (MOCNESS, Wiebe et al., 1985) in the Danish Kattegat (57°16N, 11°25E) during a cruise with the "FS Heincke" from 17 July to 7 August 1998. The hauls were kept short for 10–20 min to minimize damage of krill. Animals were sorted from the net. Krill were wrapped individually in aluminium foil and immediately placed in a −80 °C freezer. There the samples were stored until analysis.

Feeding experiments were carried out in Kristineberg Marine Research Station with animals from the Gullmarsfjord (Sweden, 58°20N, 11°34E) in late summer 1999 (20–27 September). Krill were caught with an Isaacs–Kidd Midwater Trawl (Isaacs and Kidd, 1953) from 90 m depth. The hauls lasted 5 to 10 min. The animals were immediately transferred into pre-cooled (8 °C) sea water and shipped to the laboratories.

2.2. Feeding experiments

The effects of nutrition and temperature on the concentrations of ATP and FBP were studied in laboratory experiments. Individual krill were maintained for 6 days in 1-l glass beakers in darkness. The experiments were run at 6 and 12 °C. These temperatures were the same as in the deep water (6 °C) and the surface water (12 °C) of the fjord during the experiment. Every day, half the volume of the water in the beakers was discarded and replaced by fresh fjord water taken from 35 m depth (32–34 salinity). Disturbance of animals was avoided as much as possible. One group of the animals was fed with *Artemia*-nauplii (4 days old, 1 mm long). The nauplii were concentrated over 100 μm gauze and then were added drop by drop with a pipette to the beakers with krill. The optimal number was 2000 to 3000 nauplii per animal and per day (Salomon, 2000). The second group was kept without food. At the end of the experiment (6 d) the krill were quickly grasped with a forceps dorsally in the middle of the pleon, freeze clamped, wrapped in aluminium-foil and frozen and stored in liquid nitrogen.

2.3. Tissue homogenization and purification of pyruvate kinase

Individuals were weighed to an accuracy of 0.1 mg and the sex was determined. Only animals in the intermoult stage were used. Organs and tissues were quickly dissected from frozen animals. Individuals (approx. 300 mg) or organs and tissues (40–100 mg) were homogenized with an Ultra-Turrax (Janke and Kunkel) in 2.7 ml (entire animals) or 1.2 ml (organ samples) of ice-cold extraction buffer (50 mmol $\cdot l^{-1}$ Tris/HCl, pH 7, including 60 mmol $\cdot l^{-1}$ KCl and 4 mmol $\cdot l^{-1}$ $MgSO_4$). After centrifuging at 80,000 × *g* for 30 min the supernatant was desalted and rebuffered into the elution buffer (40 mmol $\cdot l^{-1}$ Tris/HCl, pH 8, containing 4 mmol $\cdot l^{-1}$ KCl and 4 mmol $\cdot l^{-1}$ $MgSO_4$) through

Sephadex G-25 PD-10 or NAP 10 columns (AP Biosciences). Thereafter, the sample was applied to an anion-exchange column (UNO Q1-R, Bio Rad). Bound proteins were eluted with a linear gradient between 0 and 250 mmol $\cdot l^{-1}$ NaCl at a flow rate of 2.5 ml $\cdot$ min^{-1} using a FPLC-system (AP Biosciences). Fractions (0.5 ml each) containing at least 30% of the maximum PK-activity were pooled and used for further characterization.

The activity of PKI and PKII within different organs of *M. norvegica* was determined after ion-exchange chromatography. In order to obtain sufficient enzyme activity organs were pooled from several animals: eyes (4), gonads (4), hepatopancreas (4) and heart (8).

2.4. Gel filtration

The molecular weights of the two PK-isoenzymes were determined by gel filtration using a FPLC-system. The partly purified enzymes after anion exchange chromatography were applied onto a Superdex 200 HiLoad 16/60 column (AP Biosciences, 17-1069-01). The elution buffer was 0.01 mol $\cdot l^{-1}$ Imidazol/HCl, pH 6.8 including 0.15 mol $\cdot l^{-1}$ NaCl. Molecular mass calibration was performed with a gel filtration calibration kit (AP Bioscience) containing: ribonuclease A (13.4 kDa), chymotrypsinogen A (25 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), aldolase (158 kDa), catalase (232 kDa) and ferritin (440 kDa).

2.5. Enzyme characterization

PK activity was measured according to Bücher and Pfleiderer (1955) at 25 °C. Standard assays contained 500 µl extraction buffer, 20 µl NADH (6900 µmol $\cdot l^{-1}$), 20 µl phosphoenolpyruvate (PEP, 1800 µmol $\cdot l^{-1}$), 5.5 units lactate dehydrogenase (LDH) and 20 µl of the sample. The reaction was started after 5 min of preincubation by the addition of 20 µl ADP (1700 µmol $\cdot l^{-1}$). The decrease of absorbance at 340 nm was recorded photometrically.

The thermal profile of PK was determined between 5 and 55 °C. The activation energy (E_a) was calculated from the data between 5 and 25 °C by applying the Arrhenius equation.

The activities of PK isoenzymes were determined with the following effectors: ATP (167 and 2667 µmol $\cdot l^{-1}$), fructose-1,6-bisphosphate (17 µmol $\cdot l^{-1}$), and citrate, lactate, Acetyl-CoA, fructose-6-phosphate, fructose-2,6-bisphosphate, glutamic acid, glutamine, leucine, D- and L-alanine, serine, valine, threonine, cysteine, and phenylalanine (each 250 µmol $\cdot l^{-1}$). The assays were run under standard condition and at half maximum substrate concentration (33 µmol $\cdot l^{-1}$ PEP for PKI and PKII+ 200 µmol $\cdot l^{-1}$ FBP, 167 µmol $\cdot l^{-1}$ PEP for PKII and 250 µmol $\cdot l^{-1}$ ADP).

2.6. K_m-values

The K_m-values of PK for PEP were calculated from reaction velocities at substrate concentrations from 3 to 670 µmol $\cdot l^{-1}$. The concentration for ADP was kept constant at 570 µmol $\cdot l^{-1}$. The K_m-value for PKII was determined with and without FBP (200 µmol $\cdot l^{-1}$). K_m-values were calculated with the Graph Pad Prism software version 3.00 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com).

2.7. Protein determination

Soluble proteins of extracts and eluents were measured according to Bradford (1976) using a commercial protein assay (Bio Rad, 500-0006). The assay was modified for the use in microplates. In brief, 20 to 50 µl of sample were applied into the wells of the microplate and water (a. dem.) was added to a volume of 50 µl. Then 250 µl of dye solution (diluted 1:5 with a. dem.) was added. After 15 min the plate was read at 600 nm in a microplate reader. Bovine serum albumine (BioRad 500-0007, 0 to 5 µg per well) was used as a standard. On the same plate samples were applied in triplicate and standards in duplicate.

2.8. Extraction and determination of Fructose-1.6-bisphosphate (FBP) and nucleotides

Deep frozen animals were ground to a fine powder in a mortar which was cooled with liquid nitrogen. The frozen powder was transferred into 2 ml of 0.5 mol $\cdot l^{-1}$ trichloroacetic acid. It was further processed with an Ultra-Turrax T25 (Janke und Kunkel) for 20 s and incubated on ice for 10 min. After centrifugation at $4500 \times g$ for 10 min the supernatants were decanted and neutralized with trioctylamin and freon (2 ml trioc-

Table 1
Purification of pyruvate kinase from *Meganyctiphanes norvegica*

Purification step	Total protein (mg)	Total activity (U)	Specific activity (U $\cdot$ mg$_{Pr}^{-1}$)	Purification factor	Yield (%)
Crude extract	8.0	11.5	1.4	1.0	100
G25	7.7	10.1	1.3	0.9	90
UNO Q1-R	2.1	8.5	4.1	3	80
Superdex 200	0.03	1.3	42	30	11.3

Table 2
Temperature maximum, activation energy and molecular weight of PKI and PKII in *Meganyctiphanes norvegica*

Isoenzymes	Temperature maximum, °C ($n=4$)	Activation energy (E_a), kJ · mol^{-1} ($n=4$)	Molecular weight, kDa ($n=4$)
PKI	41.7 ± 1.4	53.7 ± 4.2	212.5 ± 9.4
PKII	45.0 ± 2.5	56.8 ± 2.6	235.5 ± 4.7

Significant differences between PKI and PKII were present in all parameters (Student's t-test, $p<0.05$).

tylamin 94%+10 ml freon). The aqueous phase was immediately used for FBP and nucleotide analysis. The concentration of FBP was determined as described by Michal (1984).

Nucleotides in the total body of krill were quantified by ion pair chromatography in reversed phase with a HPLC-system (Sykam, Gilching, Germany) modified after Moal et al. (1989) and Salomon (2000). Nucleotides were separated with a Phenomenex 5 µ C18 Luna-column (4.6 × 150 mm) and a same pre-column cartridge (Phenomenex, USA). The mobile phase was a NaH_2PO_4-buffer (0.2 mol · l^{-1} pH 6.0) containing 20% methanol and 5 mmol · l^{-1} tetrabutylammonium as ion-pairing agent. The flow rate was 1 ml · min^{-1}. Nucleotides were detected at 254 nm with a UV-spectrophotometer. Nucleotide standards (Sigma) of 5, 25, 50, 100 µmol were used for identification and peak quantification. The adenylate energy charge (AEC) was calculated after Atkinson (1977): AEC = (ATP + ADP/2)/(ATP + ADP + AMP).

2.9. Statistics

Data sets were analysed for statistical differences with a t-test or an ANOVA followed by the Student–Newman–Keuls test. The influence of the factors nutrition and temperature on the concentrations of ATP and FBP and on the energy charge was tested with a two way ANOVA followed by a Tukey-test. Results are presented in tables and figures as means ± standard deviation. Significant differences ($p<0.05$) are indicated by asterisks.

3. Results

3.1. Enzyme purification

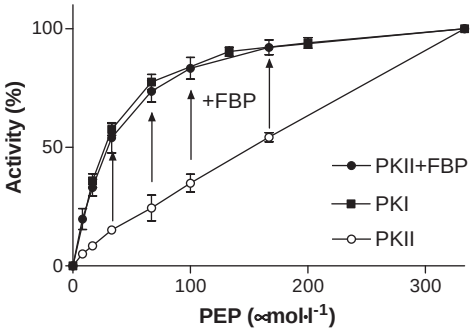
Two distinct peaks pyruvate kinase activity were separated in crude extracts of *M. norvegica*. The first enzyme (PKI) eluted at a NaCl concentration of 0.05 mol · l^{-1} and the second one (PKII) at 0.15 mol · l^{-1}. The progress of purification was similar in both isoforms. PK was enriched 3-fold after anion exchange chromatography and 30-fold after gel filtration (Superdex 200). The final yield was 11.3% (Table 1).

3.2. Molecular weight, temperature optimum and activation energy

PKI had a lower molecular weight (212.5 kDa) than PKII (235.5 kDa). Furthermore, the temperature optimum and the activation energy of PKI were slightly but significantly lower than those of PKII (Table 2).

3.3. K_m-values

The effect of PEP on the activities of PKI, PKII and PKII with 200 µmol · l^{-1} FBP was examined. The PEP saturation curves for PKI and PKII+FBP showed a strong increase in the reaction velocity when PEP concentration increased (Fig. 1). At higher substrate concentrations the curve asymptotically approached the maximum velocity (V_{max}). In contrast to this hyperbolic curve, the reaction velocity for PKII without the activator FBP was almost linear. The K_m-values for PKI and PKII+FBP were 30 and 37



Isoenzyme	K_m-value (∝mol·l^{-1})
PKI	30
PKII	1160
PKII + FBP	37

Fig. 1. The effect of PEP on the activities of PKI, PKII and PKII+200 µmol · l^{-1} FBP. The legend includes the K_m-values calculated for PKI, PKII and PKII+FBP.

$\mu mol \cdot l^{-1}$, respectively, and 1160 $\mu mol \cdot l^{-1}$ for PKII. FBP had no effect on the kinetic of PKI (Salomon et al., 2000).

3.4. Effectors

The effects of different metabolites on the activity of PKI and PKII (PKII + 200 $\mu mol \cdot l^{-1}$ FBP) were tested at half saturation concentration of the substrates PEP and ADP. ATP inhibited both isoenzymes slightly for 4–9% at a concentration of 170 $\mu mol \cdot l^{-1}$ but significantly at a concentration of 2667 $\mu mol \cdot l^{-1}$ (Table 3). The effect of ATP on the activity of PKII was the same regardless of the presence or absence of FBP. Slight activation appeared with F6P. Neither D- nor L-alanine showed distinct effects on the activities of both PK isoenzymes. This was also true for most other amino acids tested. Only cysteine and serine had a slightly enhancing effect on PKII.

3.5. Specific activity and distribution of PK

The weight-specific activity of PK in the total body of krill was higher in males than in females (61.3 $U \cdot g_{ww}^{-1}$ vs. 44.9 $U \cdot g_{ww}^{-1}$, Table 4). Furthermore, in females the share of PKII of the total activity amounted to 20%, while it was only 10% in males. The abdominal muscles had in both sexes the same ratio of PKI (90%) and PKII (10%). The specific activity in the muscles tissue of males was 50% higher than in females. Males had a higher amount of muscle tissue (49.8 ± 3.1%) than females (46.2 ± 1.6%) (*t*-test, $p = 0.004$).

The highest enzyme activity was found in the heart and in the eyes followed by the pleopods and thoracopods. Low PK-activity was found in the stomach and the hepatopancreas. PKI was the dominant enzyme in the abdominal muscle, in the pleopods, the thoracopods and in the thoracic muscle tissue. PKII, in contrast, was dominant in the gonads, the eyes and in the midgut gland. In the heart PKII amounted to 40% of the total PK-activity. The protein specific PK-activities in the different organs showed a similar pattern as the weight specific activities. However, the protein related activity in the gonads was lower because of the high concentration of soluble protein (80.6 $mg \cdot g_{ww}^{-1}$). The opposite was found in the stomach which had only 16.4 $mg \cdot g_{ww}^{-1}$ protein and, therefore, a high protein specific activity.

3.6. Nucleotides in experimentally treated animals

The concentrations of ATP ranged between 1.98 and 1.78 $\mu g \cdot mg_{ww}^{-1}$. The values of AMP and ADP were used to calculate the AEC. The AEC was similar in all experimental groups amounting to about 0.97 (data not

Table 3
The effect of different metabolites on the activity of PKI, PKII and PKII ± Fructose-1.6-bisphosphate (200 $\mu mol \cdot l^{-1}$)

Substance tested	Concentration ($\mu mol \cdot l^{-1}$)	Activity (% of a control)		
		PKI	PKII	PKII ± Fructose-1.6-bis-phosphate (200 $\mu mol \cdot l^{-1}$)
ATP	170	93.9 ± 2.1*	91.3 ± 11.6	97 ± 2.3*
	2667	4.8 ± 2.45*	27.8 ± 22.1*	28.1 ± 17.7*
Citrate	250	96.7 ± 3.6	92.5 ± 4.2*	92.5 ± 18.2
ATP ± Citrate	170 ± 250	100.1 ± 1	88 ± 3.2*	92.3 ± 4.8*
	2667 ± 250	7.6 ± 3.5*	23.4 ± 26*	16.9 ± 10*
Lactate	250	92.7 ± 3.1*	101.6 ± 6.5	93.2 ± 6.2*
Acetyl-CoA	250	93.2 ± 6.3*	86 ± 4.8*	97.4 ± 2*
Fructose-6-phosphate	250	102.3 ± 7.9	113 ± 12.2*	102.7 ± 5
Fructose-1.6-bisphosphate	17	102.3 ± 0.4*	229.3 ± 48.8*	
Fructose-2.6-bisphosphate	250	102.5 ± 3	99.6 ± 2.5	
Glutamic acid	250	98.4 ± 8.8	108.9 ± 13.5	
Glutamine	250	98.3 ± 4.3	104 ± 9.1	
Leucine	250	95.8 ± 2.6*	97.7 ± 12.2	
D-Alanine	250	99.5 ± 3.5	101.4 ± 6.4	
L-Alanine	250	98.2 ± 6.8	98.8 ± 2.7	95.6 ± 3*
Serine	250	102.3 ± 4.7	123.7 ± 16.1*	
Valine	250	96.1 ± 1.5*	97.8 ± 3.3	
Threonine	250	102 ± 3.1	106 ± 13.8	
Cysteine	250	99.3 ± 5.8	125.5 ± 9.4*	
Phenylalanine	250	100.2 ± 2.8	102.6 ± 2.8	

Values are given as percentage of the control (100%) ± S.D. Significant differences from the control are indicated by asterisks ($p < 0.05$).

Table 4
Specific activity of the PK, wet weight (ww), soluble protein and the share of PKI and PKII in the total body and different organs of *M. norvegica*

Organ	Sex	Wet weight (mg/organ)	Specific activity ($U \cdot g_{ww}^{-1}$)	Protein ($mg \cdot g_{ww}^{-1}$)	Specific activity ($U \cdot mg_{Pr}^{-1}$)	PKI (% of total activity)	PKII (% of total activity)
Total body ($n=22$)	♀	249.8 ± 47.9	44.9 ± 4.8	38 ± 3.3	1.2 ± 0.2	79.5 ± 3.3	20.5 ± 3.3
Total body ($n=22$)	♂	215.7 ± 42.7	61.3 ± 7.7	32 ± 3.6	1.9 ± 0.3	89.2 ± 2.5	10.8 ± 2.5
Abdomen without pleopods ($n=10$)	♀	102.9 ± 10.5	47.2 ± 4.7	35.6 ± 3.7	1.2 ± 0.3	89.3 ± 4.2	10.7 ± 4.2
Abdomen without pleopods ($n=10$)	♂	89.6 ± 16.7	74.1 ± 16.5	37.5 ± 6	2.1 ± 0.9	91.9 ± 2.8	8.1 ± 2.8
Pleopods and thoracopods ($n=3$)	♀	24.6 ± 2.3	54.1 ± 3.8	35.5 ± 2.3	1.5 ± 0.2	86.9 ± 3.3	13.1 ± 3.3
Thoracic muscle ($n=3$)	♀	33.1 ± 5.6	41.1 ± 9.4	47.2 ± 4.8	0.9 ± 0.4	73.6 ± 8.5	26.4 ± 8.5
"Head" (eyes, antennules, antennas, antennal scales, eye-sockets) ($n=3$)	♀	16.6 ± 2.0	51.9 ± 8.6	34.4 ± 1.8	1.5 ± 0.5	53.3 ± 7.1	46.5 ± 7.1
Eyes ($n=3$)	♀	4.2 ± 0.5	59.4 ± 8.8	56.3 ± 4.6	1.1 ± 0.1	22.8 ± 2.2	77.2 ± 2.2
Midgut gland ($n=3$)	♀	7.8 ± 0.9	16 ± 9.8	46.7 ± 25.1	0.3 ± 0.05	28.1 ± 10.2	71.9 ± 10.2
Stomach ($n=3$)	♀	2.8 ± 0.6	17.1 ± 7.7	16.4 ± 3.5	1.1 ± 0.5	56.3 ± 4.5	43.7 ± 4.5
Heart ($n=3$)	♀	1.4 ± 0.3	67.1 ± 9.1	54.6 ± 12.5	1.3 ± 0.6	60.1 ± 3.9	39.9 ± 3.9
Gonads ($n=3$)	♀	12 ± 4.4	42 ± 1.9	80.6 ± 2.7	0.5 ± 0.06	5.1 ± 3.4	94.9 ± 3.4

In routine, extracts were prepared from individual animals. If the amount of tissue was too small, samples were pooled to obtain sufficient material to analyse: eyes (4 ind.), gonads (4 ind.), hepatopancreas (4 ind.) and heart (8 ind.).

shown). Neither feeding condition nor the acclimation temperature significantly influenced the ATP content of the Northern krill.

3.7. Fructose-1.6-bisphosphate in experimentally treated animals

Both factors, temperature and nutrition, significantly influenced the FBP-concentrations in *M. norvegica* (Fig. 2). The highest amount of 6.8 $\mu g \cdot g_{ww}^{-1}$ was present in fed animals which were maintained at 12 °C. In starved animals the amount of FBP was about 30% lower then in fed animals. The same differences appeared in krill maintained at 6 °C. FBP-levels of cold acclimated animals were 35% lower then in warm acclimated specimens. Therefore, the lowest FBP concentration of 2.85 $\mu g \cdot g_{ww}^{-1}$ appeared in unfed krill maintained at 6 °C.

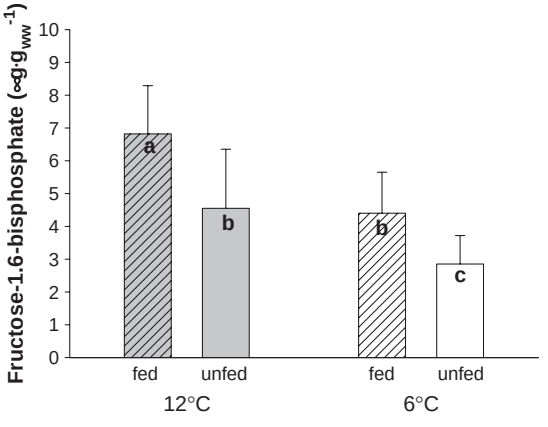


Fig. 2. Concentrations of FBP in whole body extracts of *M. norvegica* maintained at 6 and 12 °C with and without feeding. Different letters denote significant differences between treatments ($p<0.05$, $n=8$).

4. Discussion

The Northern krill, *M. norvegica*, expressed two isoforms of pyruvate kinase, PKI and PKII. Both isoforms differed distinctly in terms of chromatographic properties, thermal characteristics, activation energy and molecular masses (Salomon et al., 2000). PKI had a low K_m-value and thus a high affinity towards the substrate PEP. It was not affected by modulators. In contrast, PKII had a low affinity towards PEP. The glycolytic key metabolite FBP, however, strongly increased the affinity of PKII towards PEP. The K_m-values decreased up to 40-fold when FBP was present. In contrast, none of the tested amino acids including D- and L-alanine had significant effects on the activity of PKI and PKII. The lack of regulative potential of alanine was already reported for *M. norvegica* from the Ligurian Sea. This property was attributed to the poor anaerobic capacity of Northern krill (Spicer et al., 1999; Salomon et al., 2000). ATP inhibited both isoenzymes at high concentrations.

4.1. Organ and tissue specificity

All studied organs and tissues of *M. norvegica* contained both PK isoenzymes. However, the share of either enzyme differed strongly between the tissues and showed a distinct pattern of distribution: PKI was the predominant form in the muscles of the locomotive organs, i.e. the abdominal muscle, the pleopods, and the thoracopods. These organs also exhibited a high

specific PK-activity. It is vital for the pelagic krill to provide energy for locomotion to counteract sinking. Accordingly, it turns out plausible that PKI, the isoenzyme with the high substrate affinity, is the dominating form in the muscle tissue. In contrast, the highest share of PKII appeared in the midgut gland. This is in accordance with previous studies on several crustacean species: a PK, comparable with the mammalian L-type PK and the same properties as the PKII was found in the midgut gland of different crustaceans (Giles et al., 1977; Guderley and Hochachka, 1977; Lesicki, 1983). Additionally, PKII was the dominating isoform in the eyes and in the gonads. In the latter almost the entire PK-activity appeared as PKII. Accordingly, the glycolytic turnover in the midgut gland, the eyes and the gonads highly depends on the concentration of the activator FBP.

The FBP concentration in the tissue is correlated with the nutritional state of the animal (Pilkis et al., 1988). At limiting conditions the concentration of FBP decrease, which entails a reduction of the glycolytic energy transfer. In the gonads, energy and metabolites are mainly provided for gonad growth and maturation. Northern krill can only successfully reproduce at sufficient food supply (Cuzin-Roudy, 1993; Cuzin-Roudy and Buchholz, 1999). Accordingly, the dominance of FBP-activated PKII may be a suitable physiological mechanism to direct energy and metabolites into the gonads only when sufficient food is available.

The eyes which showed the highest specific PK activity are energy demanding organs (Demontis et al., 1997; Laughlin et al., 1998). Although metabolic costs of vision in the Northern krill are not well known yet, the energetic demand can be expected high. Due to the elevated share of PKII, a decrease in FBP-level may entail reduced glycolytic turnover rates. Thus, a reduction of glycolytic rates again may be a tool to save energy at limited nutritive conditions.

In the midgut gland the majority of metabolic processes are directly linked with food utilization. A high glycolytic turnover rate is needed particularly for synthesis of digestive enzyme and transfer processes. On starvation crustaceans reduce the size of the midgut gland and metabolize storage products such as lipids and glycogen (Strus, 1987; Papathanassiou and King, 1984). Furthermore, digestive enzyme activities decrease on starvation (Saborowski and Buchholz, 1999). The regulative potential of PKII in the midgut gland seems to be a suitable mechanism to control the glycolytic energy transfer.

The total PK-activities in the whole animals and the abdominal muscle were significantly higher in males than in females. However, males showed in the whole body 50% less PKII-activity than females. The testes are much smaller than the ovaries especially during the reproductive season (Bargmann, 1937; Albessard et al., 2001). Furthermore, the abdominal muscle was slightly larger in males than in females. Consequently, the share of PKI-rich tissue is higher in males.

4.2. Food supply and temperature

We studied the effects of nutrition and temperature on PK-kinetics by altering the levels of the major effectors ATP and FBP. In laboratory experiments *M. norvegica* were exposed for 6 days to two different feeding and thermal conditions. ATP, ADP and AMP concentrations were used to calculate the adenylate energy charge (AEC) (Atkinson, 1977). An AEC value close to 1 reflects a high amount of ATP compared to ADP and AMP. Vetter and Hodson (1982) investigated several fish species and established a "normal" value for a viable organism close to 0.9. The energy charge values published by other authors for *M. norvegica* are lower than our data. Skjoldal and Båmstedt (1976) established a maximum value of 0.76 and Saether and Mohr (1987) of 0.65. These data were gained from animals which were frozen directly after capture. These krill had most likely depleted ATP stores due to capture stress and enforced swimming. The high energy charge of 0.97 measured in our study indicates that the animals were not stressed and the handling before freezing the krill was suitable to avoid significant ATP degradation.

The ATP levels are in the same range as reported for other crustaceans (Dehn et al., 1985; Onnen and Zebe, 1983; Dickson and Giesy, 1982; Harms et al., 1990). However, studies on the influence of nutrition on ATP levels gave contradictory results, e.g. Dickson and Giesy (1982) found an increase of the ATP in the crayfish *Procambarus clarkii* and a decrease in the crayfish *Orconectes inermis* after some days of starvation. The ATP levels in Northern Krill were neither influenced by the feeding conditions of the animals nor by the temperature. Skjoldal and Båmstedt (1976) found in *M. norvegica* from the Norwegian Korsfjorden elevated ATP and adenine nucleotides levels in March and April than during the rest of the year. The authors suggested that this increase is the result of an inherent physiological rhythm associated more with the reproduction cycle than with changes in the food supply. Accordingly, it seems unlikely that pyruvate kinase is regulated by external factors via changes in the concentrations of the modulator ATP.

FBP is an activator of PKII. In *M. norvegica* the levels of FBP varied between 2.9 and 6.8 $\mu g \cdot g_{ww}^{-1}$. In comparison, Beis and Newsholme (1975) determined FBP-concentrations from 6.8 to 81.6 $\mu g \cdot g_{ww}^{-1}$ in the flight muscles of insects and a FBP-concentration of 37.4 $\mu g \cdot g_{ww}^{-1}$ in the abdominal muscle of the lobster *Homarus vulgaris*.

Food limitation and low temperatures caused a decrease in the FBP concentration in Krill. Animals which starved for 6 days showed 30% lower FBP-concentrations at both experimental temperatures. A similar reduction of FBP in the livers of rats after 10 h of food deprivation was reported by Chanez et al. (1988). The temperature also strongly influenced the FBP-levels in krill. The values for cold acclimated animals were 30% lower then those of warm acclimated ones. The decrease of FBP is correlated with the metabolic reduction at low temperatures as measured by respiration rates of krill (Saborowski et al., 2002). Accordingly, both factors influence PKII in Northern krill through the variation of FBP concentrations.

5. Conclusion

M. norvegica showed two PK-isoenzymes, an "active PK I" and "inactive PK II" one. The latter one was activated by FBP, a key metabolite of the glycolysic pathway. The concentration of FBP was highly dependent on nutrition and temperature. As a consequence of food deprivation or decreased temperature the glycolytic energy turnover may be reduced in some organs such as the gonads and the midgut gland. Simultaneously, the locomotive organs maintain high glycolytic turnover rates due to the presence of the "active" PK-isoform. The tissue specific distribution of the two different PK-isoenzymes seems to improve the krill's physiological flexibility to successfully cope with low temperatures or limited food supply.

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