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Effects of temperature on the respiration rates and the kinetics of citrate synthase in two species of *Idotea* (Isopoda, Crustacea)

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Abstract

The two species of isopods, *Idotea baltica* (Pallas) and *Idotea emarginata* (Fabricius), co-occur frequently near Helgoland, North Sea, occupying different ecological niches. Respiration rates and kinetic properties of citrate synthase (CS) were compared in these species in order to identify possible mechanisms of temperature adaptation. Specimens were acclimated to 5 and 15°C prior to further investigations. Respiration rates were measured under normoxic conditions at 5, 10 and 15°C. CS was partly purified chromatographically and influences of temperature, pH, substrate saturation and ATP-concentration on enzyme activity were examined. In both species, rising temperatures led to linearly increasing oxygen consumption, with estimated Q_{10} values between 3.2 and 4.2. Only *I. baltica* showed an effect of short term acclimation: warm adapted animals had always higher respiration rates than cold adapted ones. In *I. emarginata*, the acclimation temperature had no effect on oxygen consumption. Furthermore, its CS slightly indicates higher affinity to oxaloacetic acid when specimens were adapted to 15°C compared to those maintained at 5°C. Any effect of the experimental temperature on CS in *I. baltica* was negligible. The results are discussed in view of the different habitats occupied by the species compared. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Citrate synthase; Enzyme kinetics; Isopods; *Idotea baltica*; *Idotea emarginata*; Respiration; Temperature adaptation; Energy metabolism

1. Introduction

Adaptation to habitat-related environmental factors were reviewed in several species of crustaceans and molluscs with regard to different physiological processes (Vernberg and Vernberg, 1972). The oxygen consumption rates, the activity of metabolic enzymes and the aerobic/anaerobic capacities of three sessile invertebrates, the cirriped *Jehlius cirratus* and the bivalves *Perumytilus purpuratus* and *Mytilus chilensis* were found to be related to their vertical distribution along an intertidal rocky shore and therefore to the strong gradients of the prevailing environmental factors (Simpfendörfer et al., 1995). Temperature is the dominant abiotic factor which affects metabolic rates in poikilothermic organisms (Luxmoore, 1984). The influence of changing thermal condition on the oxygen consumption was investigated in several species (Ikeda, 1970; Du Preez, 1983; Hirche, 1984; Luxmoore, 1984). Furthermore, the activity and the kinetic characteristics of regula-

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tory metabolic enzymes were used as tools in the investigation of the adaptation potential of organisms (Somero, 1968; Mustafa et al., 1971; Ozernyuk et al., 1994; Vetter, 1995a,b; Vetter and Buchholz, 1997).

In the present study, two closely related isopod species occupying adjoining but distinctly different habitats were investigated in order to determine specific physiological and biochemical adaptations to their respective environment in particular to changing temperature. *Idotea baltica* (Pallas) and *Idotea emarginata* (Fabricius) are frequent in the North Atlantic and the adjacent North Sea where they play important roles in littoral ecosystems (Gruner, 1966; Bulnheim, 1974). While *I. baltica* mainly appears in the phytal of the upper sublittoral region or associated with drifting seaweed (Strong and Daborn, 1979; Kroer, 1986; Franke and Janke, 1998), *I. emarginata* is usually found at depths of 6–20 m, where debris of algae accumulates (Naylor, 1955a,b; Jones, 1974). Each of these habitats is exposed to different environmental factors such as light, water dynamics, oxygen supply, and thermal conditions. The temperature in the North Sea around the island of Helgoland ranges between 4 and 17°C (Radach and Gekeler, 1996). Furthermore, *I. baltica*, living close to the surface, is frequently exposed to short and medium term variations of temperature due to solar radiation. In contrast, temperatures in deeper water layers, the habitat of *I. emarginata*, are generally more stable.

The metabolic responses to short term temperature acclimation of the two species, *I. baltica* and *I. emarginata*, were compared with the aim of evaluating interspecific differences in temperature adaptation and to contribute to the understanding of physiological capabilities relevant in habitat selection. In order to obtain physiological responses on two different levels overall metabolic rates were studied by means of oxygen consumption and combined with the enzymatic characterisation of citrate synthase, a key enzyme of the Krebs cycle (Wiskich, 1980).

2. Materials and methods

2.1. Origin of animals and acclimation experiments

In order to study animals from the same genetic pool with the same environmental history *I. baltica* and *I. emarginata* were obtained from mass-cultures which were maintained separated by species under identical conditions (ambient temperature, ambient photoperiod and diet consisting of brown algae and occasionally fish) for experimental purposes at the Marine Station on Helgoland since 1989. Original material was collected at Helgoland and every year the genetic pool was supplemented with freshly caught animals from the field. Investigations were performed between May and October 1996. The water temperatures in the mass cultures were between 10 and 18°C during this time.

Individuals of both species were acclimated to cold (5°C) and warm (15°C) conditions, respectively, for at least 14 days: up to 25 animals were maintained in 5 l-tanks in temperature controlled rooms. The water was filtered (0.45 μm), continuously aerated and exchanged daily. The isopods were fed on algae (*Ascophyllum nodosum*), which also served as a substratum.

2.2. Respiration measurements

Respiration rates of cold and warm pre-adapted specimens were determined at 5, 10, and 15°C, respectively. The measurements were carried out in a closed system using cylindrical glass chambers (370 ml) with Clarke-type oxygen electrodes (Meereselektronik GmbH, Trappenkamp, Germany).

At first, the oxygen consumption at the acclimation temperature, at 5 or 15°C, was determined. Subsequently, the animals were pre-adapted to the next experimental temperature for approximately 12 h (usually over-night). In this period, they were fed on *A. nodosum*. The respiration experiments were carried out in the dark without adding food. The respiration chambers were equipped with four animals (two males and two females) and were placed in a water bath, including one chamber as a control without animals. The temperature was controlled by an external thermostat (F10VC/3, Julabo, Seelbach, Germany) with an accuracy of ± 0.1°C. In order to meet the thigmotactic behaviour of the isopods the respiration chambers were equipped with a piece of gauze. The maintenance water was filtered through 0.2 μm cellulose filters prior to the respiration experiments. Oxygen consumption

and temperature were monitored and recorded continuously with the computer program Multipar (Meereselektronik). Each respiration experiment lasted for about 4–5 h. The first hour was always used for acclimation of the animals to the experimental conditions. For calculation of the respiration rate an average of the oxygen decline in the experimental chambers from the second to the fourth hour was used. The oxygen concentration did not fall below 70% of saturation. After each experiment fresh weight (FW) and dry weight (DW) of the animals were determined. For the determination of the DW the animals were freeze-dried (freeze drier GT2, Leybold-Heraeus, Cologne, Germany). Oxygen uptake measured at 5 and 15°C was used to determine the Q_{10} after the van't Hoff equation.

2.3. Tissue homogenisation and purification of citrate synthase (CS, EC 4.1.3.7)

Four to eight specimens with a weight of approximately 2 g were homogenised with an Ultra-Turrax (Janke & Kunkel, Staufen, Germany) in 10 ml ice-cold extraction buffer (50 mmol l^{-1} Tris/HCl, pH 8.0 containing 100 mmol l^{-1} KCl and 1 mmol l^{-1} EDTA). After centrifuging at 80 000*g* for 30 min the supernatant was rebuffered into the elution buffer (40 mmol l^{-1} Tris/HCl, pH 7.0 with 20 mmol l^{-1} KCl and 4 mmol l^{-1} $MgSO_4$) by gel-filtration through Sephadex G-25 PD-10 columns (Pharmacia, Freiburg, Germany). The eluate was filtered through 0.45 µm filters (Schleicher & Schuell, Germany) and applied to a pre-packed anion-exchange column HiLoad 16/10 Q-Sepharose HP (Phamacia) equilibrated with elution buffer using a fast protein liquid chromatography (FPLC)-system (Pharmacia). The proteins bound were eluted with a linear gradient of 0–850 mmol l^{-1} NaCl within 335 ml at a flow rate of 3 ml min^{-1}. The fractions (4.3 ml each) containing more than 30% of the maximum CS-activity were pooled and used for further characterization.

2.4. Enzyme characterization

The activity of CS was measured according to Lowenstein (1969). The changes of absorbance were recorded continuously in a Lambda 2 photometer, which was equipped with a thermostated cuvette holder controlled by a peltier temperature programmer (both Perkin Elmer, Überlingen, Germany). When measuring at low temperatures (< 10°C) dry air was blown at the cuvette to avoid condensation.

The specific activity (related to wet mass) was determined at 25°C and the temperature-profile of CS in the range of 0–55°C. In order to avoid denaturation of the enzyme during the pre-incubation period at high temperatures the enzyme solution was added simultaneously when starting the reaction with oxaloacetic acid (OA).

The activation energy (E_a) of the reaction was calculated from the data of the temperature-dependent activity (0–25°C) by applying the Arrhenius equation.

The pH-dependent activity of the CS were measured between pH 7 and 9 in steps of 0.2 units at 5, 15, and 25°C. The extraction buffer was adjusted to the required pH values exactly at each of the respective incubation temperatures.

The Michaelis constants (K_m) were determined at 5, 10, 15, and 20°C for each substrate. The concentration of acetyl-CoA in the reaction mixture was chosen between 1.67 and 167 µmol·l^{-1} while the concentration of OA was kept constant at 167 µmol·l^{-1}. The K_m for OA was determined at OA concentrations between 1.67 and 167 µmol·l^{-1} with constant acetyl-CoA at 83,5 µmol·l^{-1}.

K_m values were calculated with the PC-program 'Leonora', vers. 1.0.18 by Athel Cornish-Bowden (Marseille) by iteration of the Michaelis-Menten equation.

The inhibitor constant (K_i) of ATP was determined at concentrations of 0–1670 µmol·l^{-1} ATP and calculated with the program 'Leonora' by iteration of the following formula:

$$V = \frac{V_{max} \cdot [S]}{K_m \cdot \left(1 + \frac{[I]}{K_i}\right) + [S]}$$

where V is the enzyme activity, [I] is the concentration of inhibitor, [S] is the concentration of substrate and V_{max} is the maximum turnover rate of enzyme reaction.

2.5. Statistics

For the respiration measurements the factors acclimation temperature and incubation temperature were tested using ANOVA followed by a Student-Newman-Keuls test ($P < 0.05$). The oxy-

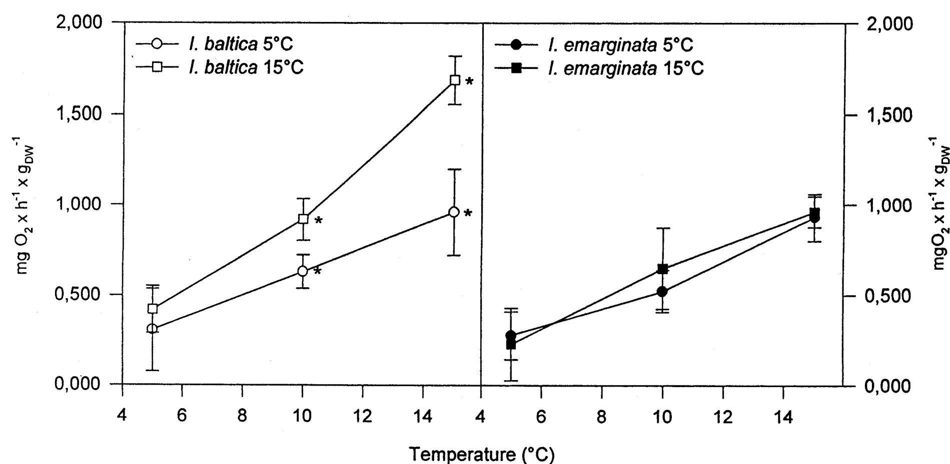

Fig. 1. (a) Oxygen consumption rates ($mgO_2 \cdot h^{-1} \cdot g_{DW}^{-1}$) of *I. baltica* and *I. emarginata* (maintained at 5 and 15°C). (b) Consumed O_2 is plotted versus incubation temperature (5, 10 and 15°C) ($n = 8$).

gen consumption of the two different species, specific activity, activation energy, K_m and K_i values from CS were compared pairwise for significant differences with a Student's t-test. Results are presented as means $\pm$ S.D. Significant differences ($P < 0.05$) are indicated by asterisks.

3. Results

3.1. Respiration

In both species, the mass specific rates of oxygen consumption increased significantly with rising incubation temperatures (Fig. 1). In cold acclimated *I. baltica* the oxygen consumption ranged between 0.3 mg h^{-1} g_{DW}^{-1} at 5°C and 0.96 mg·h^{-1} g_{DW}^{-1} at 15°C. At each incubation temperature warm acclimated specimens showed higher respiration rates than the cold acclimated ones. The values of the warm acclimated animals ranged between 0.42 mg·h^{-1} g_{DW}^{-1} at 5°C and 1.68 mg·h^{-1} g_{DW}^{-1} at 15°C. In contrast, oxygen uptake of cold acclimated *I. emarginata* did not differ significantly from those of the warm acclimated ones at any incubation temperature. In this species, oxygen consumption ranged between 0.24 mg·h^{-1} g_{DW}^{-1} at 5°C and 0.9 mg·h^{-1} g_{DW}^{-1} at 15°C. These rates corresponded to those of cold adapted *I. baltica*. In about one third of the experiments at 5°C, however, the oxygen decline of *I. emarginata* from warm and cold acclimated animals was lower by approximately 80% then the oxygen consumption rates from the other chambers under exactly the same conditions.

Q_{10} values were calculated using the respiration rates at 5°C and 15°C. In *I. baltica*. acclimated to 5°C, the Q_{10} was 3.15 while animals acclimated to 15°C showed an Q_{10} of 4.02 (Table 1). Similar results were obtained in *I. emarginata*, which had a Q_{10} of 3.35 at 5°C acclimation and 4.17 at 15°C acclimation.

3.2. Purification of citrate synthase

CS was partly purified by FPLC prior to further characterization. The chromatography from the anion exchange column Q-Sepharose HP of a crude extract from *I. baltica* and *I. emarginata* resulted in a single peak (Fig. 2). Highest activity always appeared within the NaCl-gradient at concentration of 0.2 mol·l^{-1} corresponding to fractions 25 and 26. With the described purification protocol CS was enriched $\approx$ 80-fold with a final yield of approximately 70% (data not shown).

Table 1
Q_{10} values estimated from the oxygen consumption rates of *I. baltica* and *I. emarginata*

Species	Acclimation temperature (°C)	Q_{10}
I. baltica	5	3.15
	15	4.02
I. emarginata	5	3.35
	15	4.17

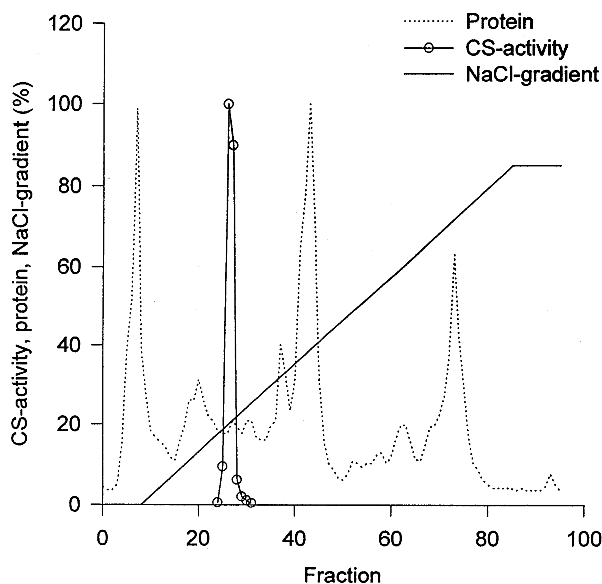

Fig. 2. Elution profile of an extract from *I. baltica* after FPLC using Q-Sepharose HP.

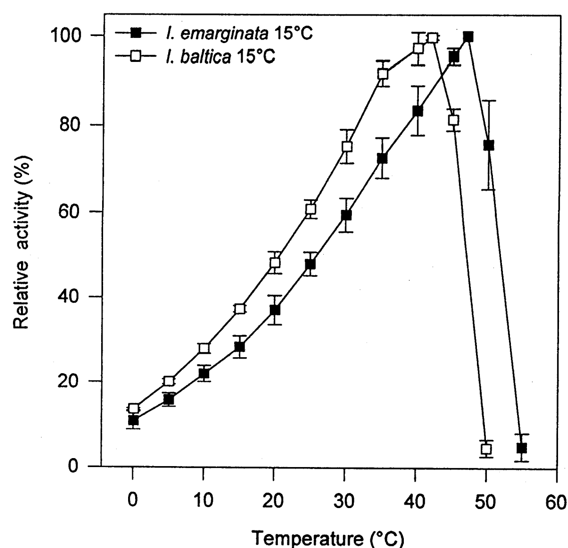

Fig. 3. Temperature dependence of CS activity from *I. baltica* and *I. emarginata* maintained at 15°C ($n = 4$).

3.3. Enzyme characteristics

There were no significant differences in the specific activity of the CS from *I. baltica* and *I. emarginata* between animals acclimated to 5 and 15°C. (Table 2). The mass specific enzyme activity was similar for both investigated isopods.

The CS of the two species showed slightly different temperature profiles (Fig. 3). The activity increased exponentially with temperature towards a maximum at 40–42°C (*I. baltica*) and 47°C (*I. emarginata*) (Table 2). Further increase of temperature (toward 55°C) caused a sharp decrease of activity to less than 5% of the maximum. Acclimation of the specimens at different temperatures had only negligible effects on temperature maxima (data not shown).

The temperature profiles from 0 to 25°C were used to calculate the E_a. In *I. baltica* acclimated to 5°C, the activation energy was 41.4 kJ mol^{-1} compared to 40.9 kJ mol^{-1} in animals acclimated to 15°C (Table 2). In *I. emarginata*, the values were similar ranging between 40.4 (5°C) and 38.7 kJ mol^{-1} (15°C). The E_a did not differ significantly between species and between acclimation temperatures within one species.

The pH profiles of CS varied depending on the assay temperatures (Fig. 4). At 5 and 15°C the activity remained stable over a wide range between pH 7 and 9 with slight decreases both at the lower and the higher limit. At 25°C, however, a distinct maximum of activity appeared between pH 7.6 and 8.2 with a steep activity decrease at higher pH values. The acclimation temperature had no effect on the pH profile of the CS in either species (data not shown). Furthermore, there were

Table 2
Specific activity, temperature maximum, activation energy and K_i values (ATP) for the CS of *I. emarginata* and *I. baltica*

Species	Acclimation temperature (°C)	Specific activity (U/g$_{ww}$) ($n = 10$)	Temperature maximum (°C) ($n = 4$)	Activation energy (E_a) (kJ·mol^{-1}) ($n = 4$)	K_i values (ATP) µM ($n = 4$)
I. baltica	5	3.1 ± 0.7	42	41.4 ± 2	842 ± 84
	15	3.1 ± 0.3	40	40.9 ± 0.2	668 ± 95
I. emarginata	5	3.4 ± 0.5	47	40.4 ± 2.3	924 ± 394
	15	2.9 ± 0.9	47	38.7 ± 1.7	956 ± 321

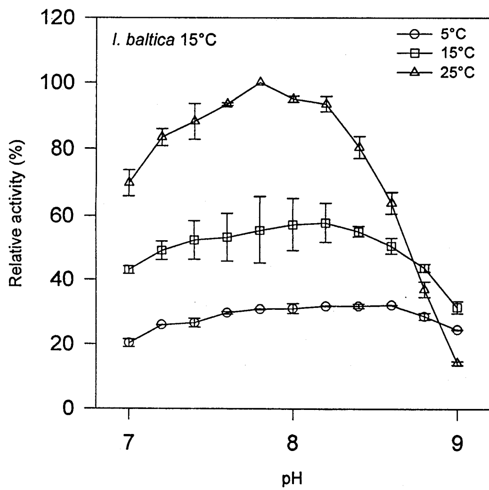

Fig. 4. PH dependence of CS activity (*I. baltica* maintained at 15°C) at 5, 15 and 25°C ($n = 3$).

no differences in the profiles of the two species.

The K_m values of CS for acetyl-CoA and OA were determined at incubation temperatures of 5, 10, 15 and 20°C. The effect of temperature on the Michaelis-Menten constants for acetyl-CoA was similar in both species (Fig. 5a,b). The K_m decreased from 5 to 10°C but remained relatively constant between 10 and 20°C. In both species, the K_m values were lower in cold acclimated animals. This effect was more evident in *I. baltica* than in *I. emarginata*. The values ranged from 14 to 23 μmol l^{-1} in *I. baltica* and 11 to 17 μmol l^{-1} in *I. emarginata*. The K_m values for OA did also decrease with temperature. This effect, however, was not as distinct as for acetyl-CoA (Fig. 5c,d). In *I. baltica*, the K_m values were almost identical in warm and cold acclimated specimens and ranged between 13 and 17 μmol l^{-1}. In *I. emarginata*, however, K_m values of cold acclimated animals were higher (15–18 μmol l^{-1}) than those of warm acclimated ones (10–13.5 μmol l^{-1}). However, this trend was only significant at an incubation temperature of 20°C due to the high

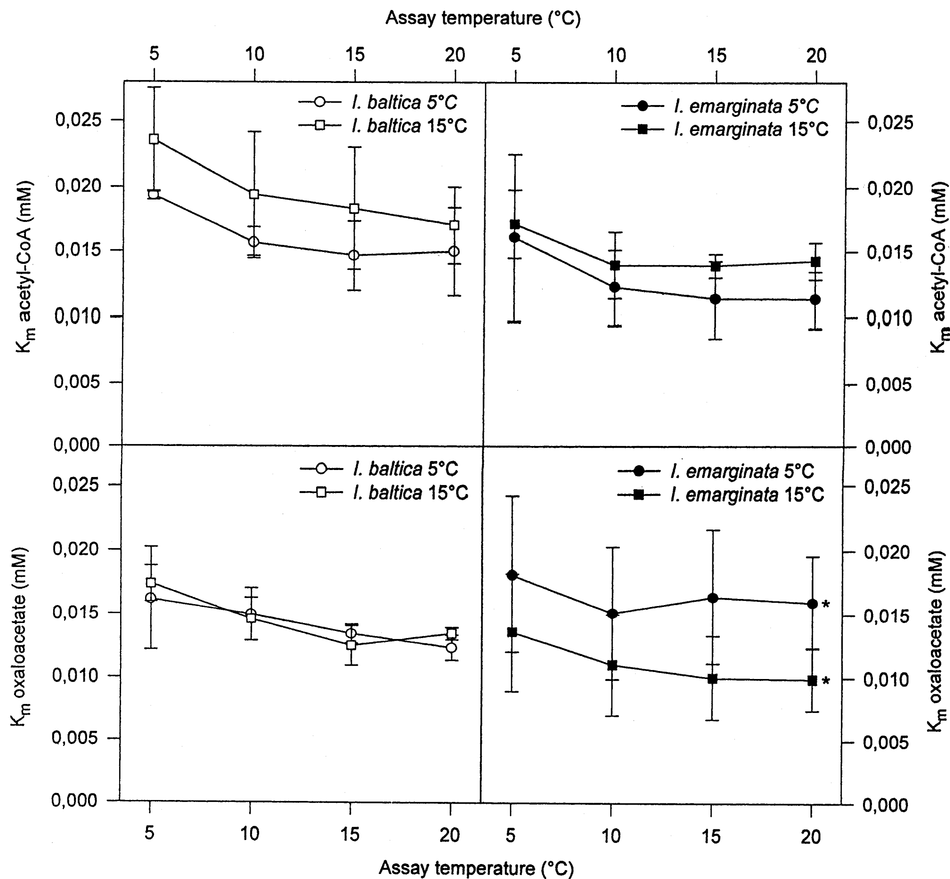

Fig. 5. Apparent K_m of acetyl-CoA (top) and oxaloacetate (bottom) of CS from *I. baltica* and *I. emarginata* ($n = 3$) ($n = 5$ for 5 d).

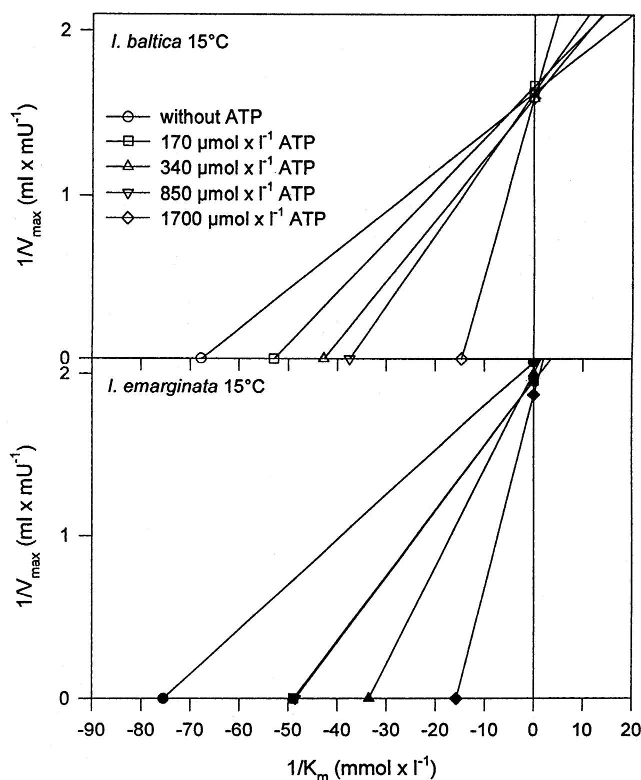

Fig. 6. Lineweaver–Burk plots of CS from *I. baltica* and *I. emarginata* maintained at 15°C. Each graph shows the calculated data of $1/K_m$ on the x-axis and $1/V_{max}$ on the y-axis.

variability of the Michaelis–Menten constants.

Furthermore, the K_m values for acetyl-CoA were estimated at different ATP concentrations. Lineweaver-Burk plots were used to define the mechanism of inhibition (Fig. 6). In all cases, competitive inhibition was noted: increasing ATP concentration caused elevated K_m values while V_{max} remained constant. The degree of competitive inhibition was quantified by the calculation of K_i values at 25°C. The K_i values were similar for both species and acclimation temperatures with 850 µmol l^{-1} in average (Table 2).

4. Discussion

Increasing temperatures from 5 to 15°C lead to 3- to 4-times higher respiration rates in both isopod species tested. A similar effect of temperature was reported by Strong and Daborn (1979, 1980) and Bulnheim (1974) in *I. baltica*. Species-specific physiological responses were, however, found in acclimation experiments. In *I. emarginata*, the experimental temperatures were not reflected in variations of oxygen uptake. Regardless of the acclimation temperature, the oxygen consumption was similar in cold and warm acclimated specimens. In contrast, *I. baltica* showed almost two fold higher rates in the specimens acclimated to 15°C. Furthermore, high acclimation temperatures lead to an increase in the Q_{10} values in *I. baltica* and *I. emarginata*, which is in accordance with Precht et al. (1973). The values calculated for the isopods maintained at 5°C were 3.1–3.3, in contrast to 4–4.2 for the warm acclimated specimens of both species. Luxmoore (1984) reported Q_{10} values for two Antarctic isopods of 2.0 in *Serolis polita* and 4.2 in *Serolis cornuta*. He explained the low value in *S. polita* to be due to a shallower bathymetric distribution and therefore to an adaptation to short-term temperature fluctuations. Generally, low Q_{10} values may be seen as an adaptation to stabilize metabolic rates (Clarke, 1983). In contrast, we found higher Q_{10} values for the warm acclimated isopods indicating a higher sensitivity for temperature changes and therefore a stronger influence of the environmental temperature on the

metabolism in these species.

Bulnheim (1974) recorded compensatory responses following thermal stress in relation to time courses of acclimation in *I. baltica*. A sudden transfer of warm acclimated animals from 15 to 5°C led to a new metabolic steady state within 3 h. In contrast, it took 15 h to stabilize a new level of metabolism after the abrupt transfer of cold acclimated specimens from 5 to 15°C. In the present study, specimens were pre-adapted for 12 h at each temperature (temperature change of 5°C) to minimize effects of rapid temperature changes and to investigate physiological responses to acute changes in temperature. After this time, the metabolic state of warm acclimated *I. baltica* was still elevated compared to the cold acclimated animals. This effect may be explained by the possibly enhanced locomotory activity of *I. baltica* in warm water. In accordance with the latter assumption, *I. baltica* showed higher swimming activities compared to other species of *Idotea* including *I. emarginata* (Tully and Ceidigh, 1986). It is possible that these findings are closely related to the distribution of the two species investigated, in their natural habitat. Bally (1987) reported that the activity potential was lowest in the isopod *Eurydice longicornis*, which occupies the lowest intertidal zone, and highest in *Excirolana natalensis*, found in the uppermost zone. The midtidal *Pontogeloides latipes* had an intermediate potential for activity. This varying scope of activity allows these animals to respond in a fine tuned way to changing conditions encountered in their milieu.

In order to investigate whether these different ways of coping with changing temperatures were also reflected on the enzymatic level, the characteristics and properties of the metabolic enzyme citrate synthase (CS) of both isopods were investigated. The temperature profiles of CS were similar in both species. However, maximum activity occurred at different temperatures, namely 42°C for *I. baltica* and 47°C for *I. emarginata*. The temperature maxima reflected the thermal stability of the enzyme (Bisswanger, 1994). The steep decrease of the enzyme activity at high temperature occurs due to thermal denaturation. Consequently, the different maxima found in both species investigated may indicate slight differences in the stability of the two enzymes due to variation in protein structure. The influence of acclimation on temperature dependent CS activity was, however, negligible.

Due to the closely matching temperature profiles, the activation energies calculated were also similar. Accordingly, temperature adaptation by reducing activation energies at low temperatures as proposed by Hochachka and Somero (1984) can be excluded in these cases. The influence of pH on CS activity was negligible at low, i.e. ambient temperatures. Since pH-regulated temperature adaptation requires distinct pH maxima (Bulnheim, 1974), pH effects appeared not to contribute to temperature adaptation of CS in these isopods. However, pH maxima did occur in CS at 25°C with steeply decreasing activity at high pH values (> 8.4) (Fig. 4). However, this was probably an effect of higher instability at alkaline pH, being both time and temperature dependent. Accordingly, the steep decrease of activity at a pH above 8.4 and a temperature of 25°C was likely due to denaturation of the enzyme and not a result of a physiologically relevant pH maximum.

Another way to maximize enzyme activity exists in the modulation of enzyme-substrate affinity (Somero, 1978; Hochachka and Somero, 1984). In our study substrate saturation curves were determined for both substrates acetyl-CoA and OA between 5 and 20°C. Michaelis constants for both substrates were in the same range, between 10 and 23 μmol l^{-1}. Furthermore, the curves did not show distinct K_m minima which could correspond to the maintenance temperature (Fig. 5). Such an effect was reported in the case of *N*-acetyl-β-D-glucosaminidase (Buchholz and Vetter, 1993) where minima of the K_m values corresponded to the respective environmental temperatures in several crustacean species from different climatic zones. In contrast to the Northern krill *Meganyctiphanes norvegica*, where pyruvate kinase is regulated seasonally by reducing the K_m values during winter (Vetter and Buchholz, 1997), K_m values of CS did not vary significantly in *Idotea*-species with one exception: the CS of the warm acclimated specimens of *I. emarginata* showed consistently lower K_m values for OA at all incubation temperatures and therefore a higher affinity to the substrate than the cold acclimated ones. However, these differences were significant only at an incubation temperature of 20°C.

Several studies reported on adaptive properties of metabolic enzymes where low temperatures lead to increasing substrate affinity as a compensating effect (Baldwin, 1971; Buchholz and Vetter,

1993; Ozernyuk et al., 1994; Vetter and Buchholz, 1997). *I. baltica* showed no acclimation effect for the substrate OA with the Michaelis constants between the values for the two experimental groups of the other species *I. emarginata*. In the latter species, however, a reverse effect was noted with increased K_m values, i.e. low substrate affinity at low acclimation temperatures.

ATP is an important inhibitor for CS activity (Phibbs and Winkler, 1982; Vetter, 1995b). The potential of regulating a metabolic enzyme by modulating the affinity to this inhibitor has been investigated in several studies (Somero, 1968; Mustafa et al., 1971; Robinson et al., 1983; Vetter, 1995b). In crustaceans from different climatic zones, Vetter (1995b) noted a substantial decrease in ATP inhibition with falling ambient temperatures. Adenylates have no effect on OA saturation kinetics when acetyl-CoA is present at high concentrations (Hathaway and Atkinson, 1965; Phibbs and Winkler, 1982). Therefore, only the inhibition of acetyl-CoA was investigated here. ATP caused competitive inhibition of the CS in both species investigated. However, in short term acclimation experiments a possible mechanism for compensating the effects of low temperature did not exist: the isopods did not show any relationship between inhibitor constants and maintenance temperatures. In general, the K_i values were comparable to those reported by Vetter (1995b).

In conclusion, in *I. baltica* the clear temperature effect on oxygen consumption (increasing maintenance temperatures enhanced the overall metabolism) was not reflected in CS. It can be suggested that the observed increase in the metabolic rate occurs due to a behavioural response to an elevated temperature regime and not as a consequence of direct metabolic adaptation processes.

In contrast, in *I. emarginata* short term acclimation did not influence respiration rates. Conversely there is only a little indication for an increase in the affinity to the substrate OA for CS at elevated temperatures pointing towards a possible down regulation of metabolic activity at low temperatures. Such an effect of down regulation however, was observed in the respiration measurements. At 5°C, in 35% of the respiration chambers the oxygen decline was much lower than in the other chambers. Apparently, several individuals fell into a state of metabolic rest at this low temperature. That this phenomenon was only observed for one third of the animals may be explained by the disturbance of the animals through their handling in preparation of the respiration experiments. During the short experimental period it may not be possible for most of the isopods to resume the state of metabolic rest.

The differences found for the two species investigated may be explained by their different life style. *I. baltica* lives in the phytal of the sublittoral or is associated with drifting seaweed (Naylor, 1955a; Salemaa, 1979; Strong and Daborn, 1979; Franke and Janke, 1998). Thereby, the species is highly exposed to diurnal and seasonal temperature changes. Furthermore, the steep increase in respiration rates indicates that rising temperatures induce swimming activity. This is necessary for the animals in their highly variable habitat. Particularly, drifting seaweeds are a substratum and food-source at the same time. They represent a limited resource and it is necessary for the animals to search actively for new substrata. Active swimming may avoid the risk of being drifted away from the coastal zone by currents and waves. Therefore, in *I. baltica* temperature changes more likely induce a behavioural response than any direct physiological effect on CS and oxygen consumption.

I. emarginata lives mostly on detritus of seaweed fragments which often concentrate in calm areas with nearly no currents at 6–20 m depth (Naylor, 1955a,b; Jones, 1974). This habitat is less affected by diurnal temperature changes. Accordingly, this species reacts differently to changing temperatures showing no behavioural response to increasing temperature. With decreasing temperature the animals can lower their metabolic rates. To maintain a down regulated metabolism appears feasible in a calm, non-exposed habitat where reduced activity has few negative consequences. Low activity in turn saves energy and may help to compensate for negative effects of cold temperatures.

These conclusions however, were not supported by our CS-determinations. Apparently, CS-activity and kinetics are not sufficiently influenced by thermal changes as to serve as a suitable indicator.

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