Marine Biology 13, 267—275 (1972)

Molecular mechanisms of adaptation to low temperature in marine poikilotherms. Some regulatory properties of dehydrogenases from two arctic species

H. W. Behrisch

The Institute of Arctic Biology, University of Alaska; College, Alaska, USA

Abstract

The properties of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from gill tissue of the tanner crab *Chionocetes bairdi*, and lactate dehydrogenase (LDH) and glyceraldehyde dehydrogenase from skeletal muscle of *C. bairdi* and the yellowfin sole *Limanda aspera* were examined over the physiological temperature range of the animals. Both animals were obtained in the Bering Sea in winter, and their enzymes appear to be remarkably cold-adapted. Affinity of sole LDH for substrate appears to increase with decreasing temperature, thus keeping reaction rate essentially independent of temperature at physiological concentrations of the substrate. Calculated values of activation energy are low, in keeping with the argument that organisms from cold environments have enzymes with a reduced energy of activation. In addition, Hill plots of the substrate saturation curves for lactate dehydrogenase from muscle of sole indicate that there is a facilitation of allosteric behaviour at low temperatures. Maximum affinity of sole LDH for substrate in the absence of univalent cations occurs at 3 °C, while in the presence of 150 mN K^+, it occurs between 0° to −2 °C. The effects of Mg^{2+} on enzyme activity appear to be determined by concentration of substrate and temperature. Thus, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are stimulated more effectively by Mg^{2+} at low temperature and at low substrate levels whereas, at high concentrations of substrate, they are relatively independent of the bivalent cation. All four dehydrogenases are affected by the univalent cations Na^+, K^+ and NH^+_4 in a manner which appears to be determined, in part at least, by concentration of substrate and by temperature. These findings suggest mechanisms for the maintenance and regulation of enzyme activity in poikilothermic tissues at low and changing temperatures.

Introduction

The dispersion into and exploitation of arctic waters by aquatic poikilotherms has been made possible by the evolutionary development of metabolic characteristics which permit the organism to maintain and control its metabolic processes in an environment of low, and sometimes widely changing, temperatures. The molecular mechanisms of temperature adaptation appear to be exceedingly complex, and a large literature on the subject has accumulated (see Hochachka and Somero, 1971, for recent review). One of the outstanding observations in recent years is that numerous physiologic functions in cold-blooded animals are insensitive to temperature (Newell, 1966, 1967; Newell and Northcroft, 1967) and, thus, there must be mechanisms which permit the animal's metabolism to remain independent of thermal changes in the environment; a number of possibilities have recently been suggested (see Behrisch, 1971).

One of the most important parameters of metabolic control is enzyme-ligand affinity. It was originally observed, in homeothermic systems, that affinity of an enzyme for its ligand (substrate, cofactor or modulator) is markedly sensitive to temperature (Helmreich and Cori, 1964; Taketa and Pogell, 1965;). Should this observation be extended to poikilotherms which experience changes in environmental temperature, it is obvious that control of metabolism in these organisms would be extremely sensitive to temperature. A generally observed effect of decreased temperature is an increase in enzyme-substrate affinity, which is undoubtedly based on a temperature-induced change in the conformation of the enzyme molecule (Iwatsuki and Okazaki, 1967). As a result, at low (and physiological) concentrations of substrate, the rate reaction at low temperature may be equal to or higher than that at higher temperatures. The relationship between temperature and enzyme-ligand interaction has been investigated for a number of enzymes from fishes, molluscs and crustaceans, and a noteworthy pattern has become discernible. For example, in acetylcholinesterase from brain of rainbow trout *(Salmo gairdneri)*, it appears that the temperature at which minimum *Km (maximum affinity)* for substrate occurs closely approximates the temperature of acclimatization of the organism (Baldwin and Hochachka, 1970). Similar results have been obtained with lactate dehydrogenases from trout, tuna, and lungfish (Hochachka and Somero, 1968), pyruvate kinases from trout, the antarctic fish *Trematomus bernachii* (Somero and Hochachka, 1968), and the Alaskan king crab *Paralithodes camtschatica* (Somero, 1969), isocitrate dehydrogenase from trout (Moon, 1970), and fructose 1,6-diphosphatase from muscle of the Alaskan king crab (Behrisch, 1971). A striking feature of these results is that, at temperatures above and below the acclimatization temperature, *Km* values

for substrate increase, resulting in a complex U-shaped temperature-*Km* curve. In many cases, the increase in *Km* is so great that, above and below the temperature of minimum *Km*, it would be very difficult for the enzyme to become saturated with substrate *in vivo*, and control would be virtually impossible. It appears that, at least in animals which experience thermal changes on a seasonal basis, acclimatization involves the synthesis of enzyme variants which are more efficient catalysts at the new temperature (see Baldwin and Hochachka, 1970; Hochachka and Somero, 1971).

In bottom waters of the Bering Sea, temperatures are likely to be relatively constant, since for a good part of the year the temperature of the water is as low as −1.8 °C and probably rises only to about 3° to 4 °C at the end of the summer. Are the enzymes of organisms of the area permanently adapted to a low temperature, to an intermediate one, or do the animals adapt to seasonal changes in temperature? To examine this question, a study was initiated on the thermal behaviour of glucose-6-phosphate dehydrogenase (G-6-P deh) and 6-phosphogluconate dehydrogenase (6-P-G deh) from the gill of the tanner crab *Chionocetes bairdi*, and two glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase (TDH) and lactate dehydrogenase (LDH) from the muscle of *C. bairdi* and the yellowfin sole *Limanda aspera*.

Materials and methods

Experimental animals

The tanner crab *Chionocetes bairdi* and the yellowfin sole *Limanda aspera* inhabit the colder waters of the northern Pacific Ocean. Temperatures in the range of distribution vary from as low as −1.8 °C in winter to as high as 4 °C (Johnson and Hartmann, 1969) and, since migrations over long distances for both these animals are not known, it is possible that they may encounter changes within −2° to 4 °C throughout the year. Crabs and sole were captured by otter trawl (with the kind assistance of Dr P. McRoy and Mr S. Stoker, Institute of Marine Sciences, University of Alaska) in the Bering Sea in the vicinity of St. Lawrence Island, Alaska, USA in waters at −1.8 °C. The animals were immediately killed, the tissues excised, and used at once in the preparation of enzyme. Tissues which were not used immediately were stored at −20 °C.

Chemical materials

All biochemical reagents used in this study were obtained from Sigma Chemical C., St. Louis, Mo., USA. The sodium salts of G-6-P, 6-P-G and pyruvate were used; 3-phosphoglyceraldehyde was obtained as the free acid in solution. All other chemicals were reagent grade.

Experimental methods

Pentose-shunt enzymes from gill of crab

Homogenate of crab gill was prepared in 10 mM tris-HCl buffer, pH 7.5 at 0 °C in a VirTis Model 23 homogenizer at top speed at 0° to 1 °C. The homogenate was spun at 3000 x gravity for 60 min at 0 °C, and the pellet discarded. This crude preparation was used to determine the total activities of G-6-P deh and 6-P-G deh. G-6-P deh was present in activities approximately 5 times as high as 6-P-G deh in the crude supernatant, and it was necessary to remove 6-P-G deh, when working with G-6-P deh, because the former enzyme, in the presence of the same coenzyme, triphosphopyridine nucleotide (TPN^+), catalyses the oxidation of the product of the G-6-P deh, reaction. The supernatant described above was brought to 55% saturation with solid $(NH_4)_2SO_4$ and stirred for at least 120 min at 0° to 1 °C. The suspension was then centrifuged at 0 °C, the pellet discarded, and the supernatant slowly brought to 70% saturation with solid $(NH_4)_2SO_4$ and stirred once more for at least 120 min at 0° to 1 °C. The precipitate was collected by centrifugation at 0 °C and taken up in a minimal volume of 10 mM tris-HCl buffer, pH 7,8 at 0° to 1 °C. The solution was then dialysed against the same buffer at 0° to 1 °C, with three changes of buffer at 4 h intervals, before use in the assays. This procedure effectively reduced activity of 6-P-G deh, to less than 5% of G-6-P deh activity, and yielded a suspension with a concentration of 10 mg protein/ml.

The other pentose-shunt enzyme examined in this study, 6-P-G deh, was prepared in a similar manner, except that it was found to be active approximately 10 times as much as G-6-P deh in the 40 to 57% $(NH_4)_2SO_4$ fraction. Dialysis of the solution was carried out as above. The reaction medium contained, in 2.0 ml of 50 mM tris-HCl buffer of different pH values, various concentrations of substrate (either G-6-P or 6-phosphogluconate), and varying concentrations of TPN^+ and $MgCl_2$. The reaction was initiated by the addition of enzyme solution to the medium and was followed by observing the rate of formation of TPNH. Unless otherwise specified, all experiments were carried out at pH 7,5.

Glycolytic enzymes from muscle of tanner crab and yellowfin sole

Homogenate of skeletal muscle was prepared in the same manner as above, spun at 3000 x gravity for 45 min at 0 °C, and the pellet discarded. The resultant solution was used directly in studies on 3-phosphoglyceraldehyde dehydrogenase (TDH) and lactate dehydrogenase (LDH). Enzyme activity was observed by following the rate of formation or oxidation of DPNH at 340 nm in a Hitachi-Perkin Elmer recording spectrophotometer.

The enzyme activities reported in this communication are the means of at least two separate determinations with variations of less than 5% between individual determinations, and are expressed as Δ OD_{340}/min/10 mg protein.

Results

Enzymes from crab gill

It is apparent that the effect of temperature on reaction rates of the two dehydrogenases from the pentose shunt in gill of the tanner crab is highly dependent on the concentration of substrate present in the medium (Figs. 1 and 2). Thus, as concentrations of both G-6-P and 6-P-G decrease, there is a drop in the temperature coefficients of the reaction, until at 0.05 mM G-6-P and 0.1 mM 6-P-G, Q_{10}, values of the reaction are near 1.0 over most of the temperature range tested; these results are quite similar to those reported for LDH's from rainbow trout, tuna and lungfish (HOCHACHKA and SOMERO, 1968). G-6-P deh does not require a bivalent cation for activity to occur, but the addition of increasing amounts of Mg^{2+} to the medium causes an increase in activity, particularly at the lower temperatures (Fig. 1). In this connection, SANWAL (1970) has reported a similar stimulation of G-6-P deh by Mg^{2+}. The response of 6-P-G deh to additions of Mg^{2+} is somewhat similar, and the enzyme appears to be stimulated more by the bivalent cation at low concentrations of substrate and at the lower temperatures ($-2°$ to 6 °C) (Fig. 2).

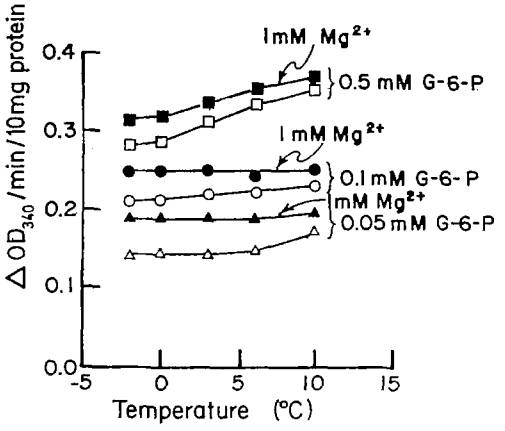

Fig. 1. *Chionoecetes bairdi*. Effect of temperature on activity of G-6-P deh at varying concentrations of Mg^{2+} and substrate. Activity = Δ OD_{340}/min/10 mg protein

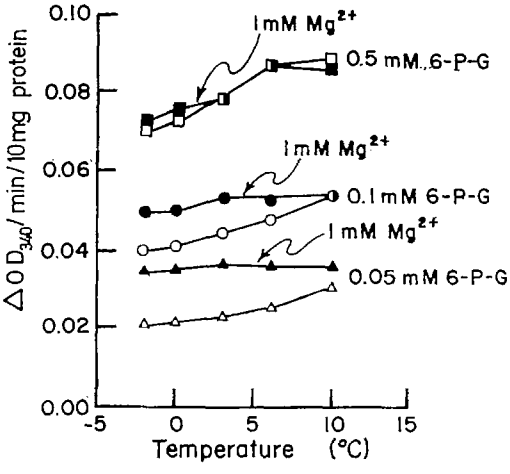

Fig. 2. *Chionoecetes bairdi*. Effect of temperature on activity of 6-P-G deh at varying concentrations of Mg^{2+} and substrate. Activity = Δ OD_{340}/min/10 mg protein

Glycolytic enzymes from muscle of crab and yellowfin sole

Lactate dehydrogenase (LDH) from muscle of yellowfin sole does not appear to display a marked temperature optimum at any concentration of substrate, and the results yield correspondingly low values of activation energy (Table 1). However, it is interesting to note that the shape of the substrate saturation curve changes with temperature; thus as temperature is increased from $-2°$ to 3 °C, there is a shift from a hyperbolic curve to a sigmoid one (Fig. 3), accompanied by an attendant increase in the values of the HILL constants (n_H), indicating an increase in homotropic cooperation in the binding of substrate. At 6° and 10 °C, the saturation curve is again hyperbolic. This apparent change in the allosteric behaviour of the enzyme molecule is accompanied by an increase in affinity for pyruvate as temperature rises from 0° to 3 °C. These results yield a U-shaped temperature-*Km* curve, reminiscent of those reported for isocitrate dehydrogenase from the trout (MOON, 1970) and fructose diphosphatase from muscle of the Alaskan king crab (BEHRISCH, 1971). An interesting result is observed when pyruvate saturation curves are carried out in the presence of 150 mM K^+; the U-shaped *Km*-temperature curve disappears and the minimum apparent *Km* for pyruvate now falls near the temperature of acclimatization of the animals used in the present experiments (Fig. 3). It is also known that changes in pH can alter the kinetic behaviour of LDH (VESELL, 1966; VESELL and YIELDING, 1966), and an experiment was performed on the sole LDH in which the pH of the medium was changed in accordance with the observations of REEVES and WILSON (1969). A rise in pH at the lower temperatures thus results in a shift from a hyperbolic saturation curve to a sigmoid one (Fig. 4), accompanied by an increase in the HILL constants (Table 1).

In agreement with the observation that muscle of the king crab has high activities of glycolytic enzymes (HOCHACHKA et al., unpublished results),

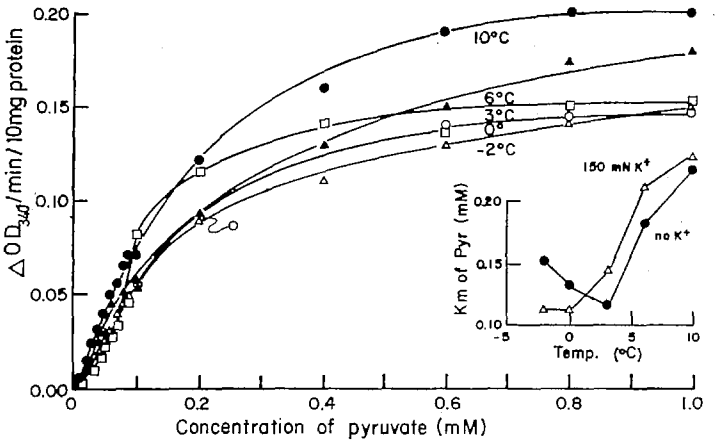

Fig. 3. *Limanda aspera.* Substrate saturation curves of sole muscle LDH at varying temperatures. Inset: effect of temperature on *Km* of pyruvate in presence and absence of K^+ ion. Activity = $\Delta\ OD_{340}$/min/10 mg protein

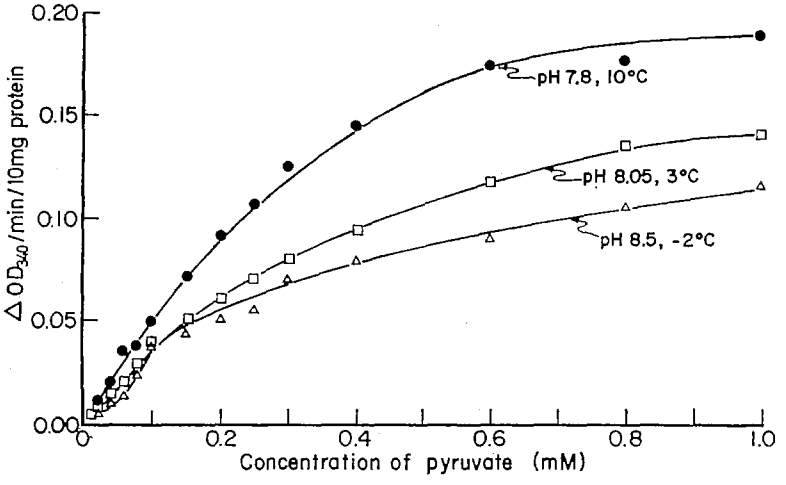

Fig. 4. *Limanda aspera.* Substrate saturation curves of sole LDH in which pH of medium was varied with temperature (see text for explanation). Activity = $\Delta\ OD_{340}$/min/10 mg protein

Table 1. *Limanda aspera. Effect of temperature on affinity of sole muscle LDH for pyruvate, n_H values and activation energy (calculated from V_{max})*

Temperature (°C) (constant p_H)	*Km* of pyruvate (mM) (no K^+)	(150mN K^+)	n_H (150mN K^+)	Probability of n_H being zero	Temperature range (°C)	Activation energy (kcal/mole)
−2°	0.15	0.11	1.3	0.000	−2°— 0°	0.00
0°	0.13	0.11	1.2	0.000	0°— 3°	0.00
3°	0.11	0.15	2.0	0.000	3°— 6°	9.4
6°	0.18	0.21	1.3	0.000	6°—10°	4.0
10°	0.24	0.22	1.5	0.001		

Temperature (°C)	*Km* of pyruvate (mM)	Temperature range	Activation energy (kcal/mole)
−2°	0.26	−2°— 3°	5.8
3°	0.26	3°—10°	6.8
10°	0.17		

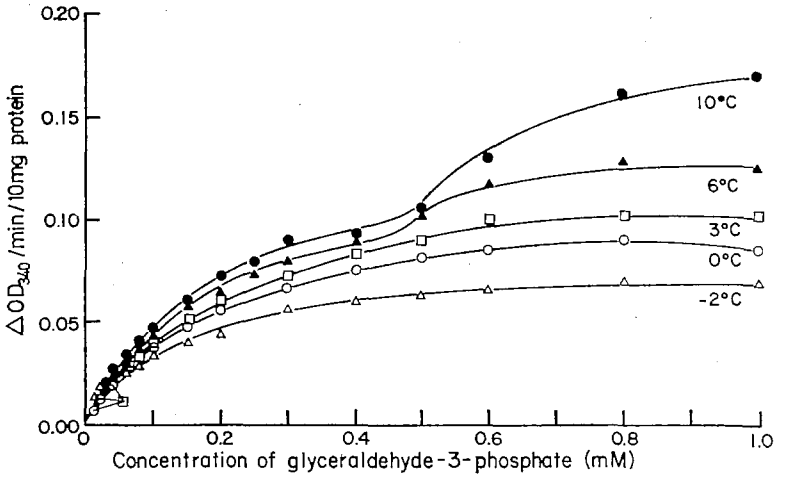

Fig. 5. *Chionocetes bairdi*. Substrate saturation curves of tanner crab 3-phosphoglyceraldehyde dehydrogenase at series of temperatures

Table 2. *Chionocetes bairdi. Effect of varying concentrations of Na^+, K^+ and NH^+_4 on activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from gill of tanner crab*

Concentration of substrate (mM)	Concentration of cation (mN)	Relative activity (% of control)								
		Na^+			K^+			NH^+_4		
		−2°C	3 °C	10 °C	−2 °C	3 °C	10 °C	−2 °C	3 °C	10 °C
0.05 G-6-P	0	100	100	100	100	100	100	100	100	100
	10	125	110	120	115	108	106	112	110	105
	50	150	140	135	137	126	120	163	149	146
	100	200	175	176	167	143	139	197	186	170
	150	250	240	212	196	160	159	239	234	216
0.1 G-6-P	0	100	100	100	100	100	100	100	100	100
	10	105	104	103	116	109	107	113	108	103
	50	141	137	143	143	129	118	159	147	126
	100	186	182	179	171	146	136	189	188	139
	150	234	226	222	191	159	150	234	231	178
0.5 G-6-P	0	100	100	100	100	100	100	100	100	100
	10	103	101	100	103	102	100	115	111	103
	50	126	117	105	130	125	113	163	156	129
	100	153	137	119	161	146	129	194	184	153
	150	184	158	134	186	160	145	230	216	176
0.05 6-P-G	0	100	100	100	100	100	100	100	100	100
	10	105	120	115	113	111	109	113	117	100
	50	150	150	150	131	127	130	162	160	96
	100	200	190	195	149	143	149	195	197	83
	150	265	235	240	162	154	164	230	230	61
0.1 6-P-G	0	100	100	100	100	100	100	100	100	100
	10	104	103	101	110	110	108	109	106	94
	50	143	124	119	134	133	131	156	137	76
	100	189	149	136	148	146	148	189	168	68
	150	227	176	153	160	157	163	229	199	64
0.5 6-P-G	0	100	100	100	100	100	100	100	100	100
	10	100	101	102	109	107	106	108	105	100
	50	123	116	114	126	120	121	151	136	101
	100	146	132	125	143	136	135	186	161	192
	150	164	149	130	151	144	143	224	178	84

Table 3. *Effects of univalent cations on activity of glyceraldehyde phosphate dehydrogenase (TDH) from muscle of yellowfin sole (Limanda aspera) and tanner crab (Chionocetes bairdii) at different temperatures*

	Relative activity (% of control)								
	Na^+			K^+			NH^+_4		
L. aspera THD Concentration of cation (mN)	−2 °C	3 °C	10 °C	−2 °C	3 °C	10 °C	−2 °C	3 °C	10 °C
0	100	100	100	100	100	100	100	100	100
10	116	120	105	113	114	106	116	116	113
50	131	143	118	140	136	119	136	132	127
100	126	121	103	178	159	134	156	148	145
150	97	98	91	201	178	149	173	161	159
C. bairdi THD Concentration of cation (mN)									
0	100	100	100	100	100	100	100	100	100
10	103	106	107	115	103	100	100	105	104
50	126	127	125	143	107	94	106	124	123
100	140	149	139	182	111	90	113	136	137
150	121	116	109	217	115	83	120	150	148

muscle of *Chionocetes* has high activities of 3-phosphoglyceraldehyde dehydrogenase (TDH). Like TDH's from other sources (Nordin et al., 1970), the enzyme from the tanner crab has rather a low affinity for substrate, glyceraldehyde phosphate (Fig. 5). An interesting characteristic of the crab TDH is the markedly biphasic substrate saturation curve at the higher (6° to 10 °C) temperatures, similar to that noted by Nordin et al. (1970) in purified honeybee TDH. Such biphasic saturation curves have been also noted in cofactor (Mg^{2+}) saturation curves in fructose diphosphatase from migrating salmon (Behrisch, 1969) and pyruvate kinase from muscle of the Alaskan king crab (Somero, 1969). Somero suggested the existence of thermal isomers of the pyruvate kinase enzyme molecule, while Gelb et al. (1970) have suggested that the changing nature of the saturation curve of purified honeybee TDH is due to an interaction of the effects of substrate and cofactor on the conformational state of the enzyme molecule.

Effects of univalent cations on enzyme activity

Numerous publications report the activation of enzyme activity by the univalent cations Na^+, K^+ and NH_4^+ (for a comprehensive list see Dixon and Webb, 1964). The three cations have been shown to affect the activity of dehydrogenases from a wide variety of sources, with K^+ and Na^+ usually being the most effective modulators. The effectiveness of these cations in influencing activities of the dehydrogenases in the present study appears to be determined by temperature as well as concentration of substrate and cofactor (see also Behrisch, 1971). Thus Na^+ stimulates G-6-P deh and 6-P-G deh about equally at all temperatures, and at Vmax concentrations of substrate (Table 2). However, as the concentration of substrate is decreased, there is greater stimulation of the activities of both pentose shunt enzymes. NH_4^+ stimulates both G-6-P deh and 6-P-G deh equally at the two lower temperatures (−2° and 3 °C) at all levels of substrate used. However, at 10 °C, NH_4^+ appears to act as a weak inhibitor of 6-P-G deh, while it continues to stimulate G-6-P deh activity, although less effectively than at the two lower temperatures (Table 2).

The response to univalent cations of the two TDH's from muscle of crab and sole is less uniform (see Table 3). TDH from sole is activated by Na^+ approximately equally at all three temperatures tested while, on the other hand, high concentrations of Na^+ appear to cause a decrease in activity of the TDH from the crab (Table 3). NH_4^+ appears to enhance activities of the crab and sole TDH's at 3° and 10 °C while, at −2 °C, the crab enzyme appears to be largely independent of NH_4^+ and the TDH from the sole is stimulated by the cation at −2 °C, the temperature of acclimatization of the organisms used in this study. K^+ ion stimulated the crab TDH at −2 °C while, at 3 °C, the enzyme is largely independent of the cation. Finally, at 10 °C, K^+ acts as an inhibitor of TDH activity in the crab, an effect similar to that of K^+ on activity of fructose diphosphatase from muscle of the king crab (Behrisch, 1971). Thus, the ability of K^+ to act either as an activator at low temperature or as an inhibitor of enzyme

activity at the higher physiological temperatures indicates that the cation could render TDH activity virtually independent of temperature.

Discussion

A special manifestation of metabolic control in poikilotherms is seen in an adjustment of metabolism in compensation to changes in ambient temperature. It is generally agreed that such temperature compensation of metabolism can be genetically determined (see SCHOLANDER et al., 1953; PROSSER, 1957) or environmentally induced (BALDWIN and HOCHACHKA, 1970). Although few data are available on the underlying mechanisms, these two processes of temperature compensation are usually considered distinct (see PROSSER, 1957), and it follows that the environmentally-induced temperature compensation will occur within the bounds imposed by the extent to which the animal's genetic complement "anticipates" the environment. Some previous attempts to understand metabolic compensation in evolutionary adaptation to temperature have considered: (1) activation energies (see VROMAN and BROWN, 1963); (2) thermal optima for maximal velocities (see READ, 1964). The present data (Table 1) for the sole and tanner crab from Bering Sea waters in winter, give exceedingly low calculated values for activation energy (E_a), in keeping with the argument of VROMAN and BROWN that enzymes of organisms from environments of low thermal energy have low calculated E_a values to compensate for the low thermal energy available. Previous data (HOCHACHKA and SOMERO, 1968; BEHRISCH, 1969; BEHRISCH and HOCHACHKA, 1969), for animals from temperate environments, do not show any consistent trend within this hypothesis, but perhaps one would not expect selection for enzymes with low (or high) E_a values in organisms from such environments. At any rate, work in this laboratory on numerous enzyme systems from arctic and subarctic poikilotherms (BEHRISCH, 1971; BEHRISCH, unpublished data) shows that, in these cases, the calculated values of E_a are very low indeed.

An interesting feature of the present data is the observation that temperature appears to affect affinity of enzyme for substrate(s) (Table 1; Figs. 1, 2, 3). Thus, at low (and physiological) concentrations of substrate, a drop in temperature will cause an increase in catalytic activity, which will effectively counter the decrease in thermal energy, which in turn would tend to slow down the rate of reaction. Similar results have been observed for the pyruvate kinases of the Antarctic fish *Trematomus* (SOMERO and HOCHACHKA, 1968) the Alaskan king crab (SOMERO, 1969), isocitrate dehydrogenase (MOON, 1970), and acetylcholinesterase from rainbow trout (BALDWIN and HOCHACHKA, 1970), and fructose diphosphatase from muscle of the Alaskan king crab (BEHRISCH, 1971). In these studies, it was observed that minimum *Km* for substrate (maximum affinity) occurred at a temperature which closely coincided with the acclimatization temperature. In this connection, it is observed that, in the LDH from muscle of sole, and in the absence of any monovalent cation, maximum affinity for substrate (pyruvate) occurs at around 3 °C (Table 1), whereas the fish used in these studies were caught in waters at −1.8 °C. In an identical experiment, but in the presence of 150 mM K^+, minimum apparent *Km* of the enzyme for substrate now occurs at around −0.5 °C, very near the temperature of acclimatization of the organisms used in these studies.

The ability of the monovalent cations Na^+, K^+, and NH_4^+ to inhibit or stimulate selectively the activities of G-6-P deh, 6-P-G deh, LDH and TDH from two species, and in a manner determined, in part at least, by concentration of substrate and cofactor as well as temperature (see Tables 2 and 3) suggests that these ions may have a role in the regulation of enzyme activity and metabolism. HUBERT et al., (1970) and BEHRISCH (1971) observed that these cations can alter the activities of gluconeogenic enzymes from a variety of fishes and crustaceans. In addition, although the importance to ionic regulation of gill tissue in the Crustacea is widely recognized (FLEMISTER, 1959), the rather obvious relationship between cations and activities of gill enzymes has hardly been examined. In studies on homogenate of gill from the Alaskan king crab, HOCHACHKA et al., (unpublished data) noted that Na^+ activated oxidative reactions, Ca^{2+} inhibited and K^+ was relatively ineffective. In addition, Mg^{2+} appeared to inhibit the activity of the pentose shunt in gill homogenate, although the activity of 6-P-G deh was stimulated, a finding supported in the present study (Fig. 2). The probable importance of cations in control of enzyme activity is further underscored by the observations that Na^+ enhances the activities of both G-6-P deh and 6-P-G deh from crab gill at low substrate concentrations, and that K^+ ion could maintain activities of crab TDH and sole LDH virtually independent of temperature (Table 2, Fig. 1). These findings are of particular relevance to metabolic control in poikilotherms because in addition to those of a number of bivalent cations, the concentrations of Na^+ and K^+ vary during thermal acclimatization in poikilotherms (RAO, 1962; HICKMAN et al., 1964; TOEWS and HICKMAN, 1969; UMMINGER, 1969, 1970). Changes in the concentrations of these cations and compartmentation are known to occur in the cell (BYGRAVE, 1966, 1967) and, judging from the present results (Tables 2 and 3), such changes in cation concentration may well be a major method of influencing enzyme and metabolic activity.

Further, it is known (ROBIN, 1962; RAHN, 1965; REEVES and WILSON, 1969) that temperature-caused changes in intracellular pH occur in poikilotherms. A decrease in temperature results in a rise in intra-

cellular pH, which parallels a rise in the ionization constant of water at low temperature (see Rahn, 1965, for discussion). In an experiment in which pH and temperature were varied in accordance with the observations of Reeves and Wilson (1969), it was found that the normally hyperbolic substrate saturation-curve for muscle LDH from sole became quite markedly sigmoid (Fig. 4), suggesting homotropic cooperativity of the subunits and, as a result, a smaller change in substrate concentration would be needed to effect a change in catalytic activity.

It seems not unreasonable to accept that the regulatory properties of enzymes confer selective advantage to the cell and organisms only when they are effective under physiological concentrations of substrate, cofactor and modulator. Therefore, it appears that the observed effects on enzyme-substrate interaction (see Fig. 3) within the physiological thermal range of the animal offer a formal mechanism for the regulation of enzyme activity. It is also evident that a number of presumably adaptive characteristics of an enzyme molecule may not become apparent unless the enzyme is examined under various and physiologically relevant conditions.

Summary

1. The effect of temperature on the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase from gill tissue of the tanner crab *Chionocetes bairdi*, and lactate dehydrogenase and glyceraldehydephosphate dehydrogenase from muscle of *C. bairdi* and the yellowfin sole *Limanda aspera*, is highly dependent on the concentrations of substrate, cofactor and selected cations.

2. The activities of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase from gill of the crab are relatively independent of temperature at low concentrations of substrate. Mg^{2+} appears to stimulate activity of both G-6-P deh and 6-P-G deh, particularly at lower temperatures.

3. Maximal velocities of lactate dehydrogenase and glyceraldehydephosphate dehydrogenase from muscle of the tanner crab and the yellowfin sole appear to be largely independent of temperature, and calculated values of activation energy are very low. Affinity of both enzymes for substrate increases with decreasing temperature, and both lactate dehydrogenase and glyceraldehydephosphate dehydrogenase seem to undergo a kinetic transition which is reflected in the shape of the substrate saturation curves.

4. The univalent cations Na^+, K^+, and NH_4^+ are able to act as stimulators or inhibitors of the activities of the above enzymes, in a manner which is determined, in part at least, by concentrations of substrate, cofactor and temperature.

5. These findings suggest mechanisms for the maintenance and regulation of enzyme activity in arctic poikilotherms at low temperatures.

Acknowledgements. This study was supported by Grant number GB 20476 from the National Science Foundation, and carried out on board the USCGC "Glacier" during the 1971 cruise in the Bering Sea ice. The author wishes to thank the officers and men of the "Glacier", without whose kind help and cooperation this work would not have been possible, and Dr. P. Hochachka for his critical reading of the manuscript.

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Author's address: Dr. H. W. BEHRISCH
The Institute of Arctic Biology
University of Alaska
College, Alaska 99701
USA

Date of final manuscript acceptance: November 19, 1971. Communicated by J. BUNT, Miami