Comp. Biochem. Physiol., 1964, *Vol.* 12, *pp.* 331 *to* 340. *Pergamon Press Ltd. Printed in Great Britain*

THE TEMPERATURE DEPENDENCE OF MYOSIN-ADENOSINETRIPHOSPHATASE AND ALKALINE PHOSPHATASE IN LIZARDS*

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(*Received* 23 *December* 1963)

Abstract—1. The temperature dependence of myosin ATP-ase and intestinal alkaline phosphatase from a variety of lizards having distinct preferred temperatures was compared.

2. The ATP-ase was relatively thermolabile; reductions in activity at temperatures above the optimum resulted primarily from irreversible denaturation. However, pronounced differences were evident in both the optimal temperatures and thermostabilities of the ATP-ases from eight species of lizards. Optima ranged from 33–42°C.

3. Alkaline phosphatase was more heat resistant and reductions in activity above the optimum were largely reversible. There was virtually no difference in the temperature dependence of the alkaline phosphatase from four species studied. The optimal temperature for this enzyme was about 42°C and no denaturation was evident at 51°C.

4. Differences in the temperature dependence of the ATP-ase correlated well with differences in the preferred temperatures of the respective species. Thus, biochemical adjustments in ATP-ase appear to be closely associated with thermal adaptations evident at the level of the whole animal; but it is clear that not all enzymes are similarly involved in organismal thermal adjustments.

INTRODUCTION

UNDER favorable conditions, lizards tend to maintain body temperatures within relatively narrow limits by behavioral means. Pronounced differences in the characteristic "preferred temperatures" or "thermal preferenda" at which lizards thermoregulate are evident among species, even in the same habitat (Bogert, 1949). Available information on the physiological responses to temperature in lizards indicates that these interspecific differences in thermal preferenda are associated with physiological adjustments to different temperatures (Dawson & Templeton, 1963; Licht, 1964). However, the mechanisms involved in the evolution of these adjustments are still only poorly understood.

* Work supported in part by grant G-23137 from National Science Foundation to Dr. W. R. Dawson, and NSF Cooperative Graduate Fellowships for the years 1961–62 and 1962–63 to the author.

This paper is part of a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree at the University of Michigan.

In view of the importance of enzymatic reactions to the temperature dependence of physiological processes, biochemical adjustments with respect to temperature may play an important role in the environmental thermal relations of organisms. Indeed, seemingly adaptive differences in the thermostabilities of several enzymes have been demonstrated in closely related species of fish and of frogs from different thermal environments (Kusakina, 1963).

The present investigation was undertaken to determine whether species differences at the subcellular level are associated with the diversity in the preferred temperatures of lizards. Observations are presented here on the temperature dependence of myosin-adenosinetriphosphatase (ATP-ase)* and intestinal alkaline phosphatase from nine species of lizards.

MATERIALS AND METHODS

Procurement and maintenance of lizards

A list of the sources of the various species used in this study is provided in Table 1. Enzyme determinations were made as soon as possible after the capture

TABLE 1—SOURCES OF EXPERIMENTAL ANIMALS

Family and species	Source
AGAMIDAE	
Amphibolurus ornatus	Perth, Western Australia
ANGUIDAE	
Gerrhonotus multicarinatus	Riverside County, California†
GEKKONIDAE	
Gymnodactylus milii	Perth, Western Australia
IGUANIDAE	
Anolis carolinensis	Harris County, Texas
Dipsosaurus dorsalis	Riverside County, California
Sceloporus undulatus	Crawford County, Missouri
Uma notata	Imperial County, California
SCINCIDAE	
Egernia carinata	Perth, Western Australia
Eumeces obsoletus	Kansas†

† Supplied by commercial collector.

of the lizards, usually within several weeks. In the laboratory, these animals were kept in wooden cages fitted with incandescent lamps which supplied both light and heat for 12 hr daily. Measurements of body temperatures in these cages indicated that the species continued to thermoregulate at the same level as when freshly captured. This was taken as evidence that little if any thermal acclimation with

* The following abbreviations will be used: ATP (adenosine triphosphate); ATP-ase (adenosinetriphosphatase).

respect to preferred temperatures occurred during the period of confinement. The diet of animals in the laboratory was varied according to the habits of the species. With the exception of *Dipsosaurus dorsalis*, most were given primarily mealworms. The *Dipsosaurus* were maintained on prepared dogfood and lettuce.

Myosin ATP-ase

Preparation of homogenates.—Homogenates were prepared from a combination of the limb and dorsal trunk musculature of each lizard. Myosin was extracted from the minced muscles for 15 min with an acid phosphate-KCl solution (Guba-Straub) containing 0·015 M ethylenediamine–tetraacetate to remove endogenous calcium. The extract was precipitated by addition of 10 vol of water. The precipitate was dissolved in 0·6 M KCl, clarified by centrifugation ($3000 \times g$ for 10 min) and reprecipitated by dilution. All measurements were completed within 72 hr.

Measurement of activity.—The precipitated myosin was diluted with 0·06 M KCl to obtain ATP-ase activity in a convenient range for measurement. Preliminary tests indicated that the variations in concentration of myosin within the range used did not affect the temperature dependence of the enzyme. To measure the effects of temperature on ATP-ase activity, 0·2 ml of the dilute myosin was mixed with 0·9 ml of substrate solution which contained sodium ATP from Pabst Laboratories (5 mM) buffered with tris (hydroxymethyl) aminomethane (5 mM) at pH 8. After this mixture had been equilibrated at a desired temperature for 7 min, 0·1 ml of 1 mM $CaCl_2$ was added to start the reaction; the hydrolysis of ATP by the myosin was negligible in the absence of $CaCl_2$. Pairs of duplicate tubes were then incubated for 7 and 22 min; the reaction was stopped at the appropriate time by addition of 0·4 ml of 30% trichloroacetic acid. ATP-ase activity was determined by measuring the amount of inorganic phosphate liberated in the last 15 min of incubation. Inorganic phosphate was measured colorimetrically by the following modification of the method of Fiske & SubbaRow (1925): a 1·0 ml aliquot of the reaction mixture was incubated for 20 min with 0·1 ml of 4% ammonium molybdate (dissolved in 8 N H_2SO_4) and 1·0 ml of 0·05% N-phenyl-p-phenylenediamine (dissolved in 1% $NaHSO_3$). The optical density of the solution was then measured with a Coleman Model 6A spectrophotometer at 700 mμ. To facilitate comparison of homogenates of different specific activities, the ATP-ase activities at various temperatures were expressed as a percentage of the maximal activity measured for each homogenate.

Measurements were also made of the extent of irreversible denaturation at various temperatures above the optima to gain insight into the basis for changes in activity at these upper temperatures. A sample of dilute myosin with buffered ATP (see proportions above) was incubated for 15 min at a given temperature. At the end of this period, the mixture was cooled on ice for 10 min and then rewarmed to 20°C. $CaCl_2$ was then added to the warmed sample to start the reaction and the amount of substrate split in 20 min was determined. The difference between the ATP-ase activity of these preheated samples and that of a control sample which was

not preheated provided an estimate of the extent of irreversible thermal denaturation that occurred during the 15 min incubation at the upper experimental temperature. This aspect of the temperature dependence of the enzyme is referred to as thermostability.

Alkaline phosphatase

Preparation of homogenates. The small intestine was removed from a lizard and the contents were emptied by stripping with forceps. The intestinal tissue was minced and homogenized in cold distilled water. Homogenates were frozen and thawed six times. Measurements of enzymic activity were completed within 1 week, although no measurable change in activity was evident in homogenates stored frozen for 6 months.

Measurement of activity. Intestinal homogenates were diluted with distilled water to reduce alkaline phosphatase activities to levels convenient for measurement. Preliminary tests showed that the optimal pH for this enzyme varied from 9·0 to 9·5 for the four species studied. However, the nature of the temperature dependence was unchanged between pH 8·5–10·0. Measurements were subsequently made at pH 9·0.

The reaction was started by adding 0·2 ml of the dilute homogenate to 2·0 ml of preheated substrate solution (11 mM Na β-glycerophosphate, 12 mM H_3BO_3–NaOH buffer, and 1 mM $MgSO_4$). After incubating for 25 min at the desired experimental temperature, the reaction was stopped by addition of 0·4 ml of 30% trichloroacetic acid. Activity was determined by measuring the amount of inorganic phosphate liberated using the method described above. Appropriate blanks were used to correct for free inorganic phosphate present in solutions prior to the incubation period.

For estimates of thermostability, a sample of homogenate was incubated for 15 min (without substrate) at 51°C, cooled on ice and rewarmed to 20°C. The activity remaining was then measured and compared with controls as described for ATP-ase.

RESULTS

Myosin ATP-ase

The relative activities of myosins at various temperatures and the extent of irreversible denaturation in the upper end of the same range of temperature are illustrated in Fig. 1. Activity increases with temperature up to a maximum and then declines abruptly at higher temperatures. The slopes of the curves for relative activities below 30°C correspond to a Q_{10} of 1·7, indicating an activation energy of *ca.* 9000 cal/mole. This latter value is very similar to that reported for precipitated myosin from rabbit psoas muscle at comparable temperatures (Brown *et al.*, 1958).

There is a close correspondence between the temperature at which irreversible denaturation commences in each species and the temperature at which relative activity is maximal (the optimal temperature). This relationship indicates that the

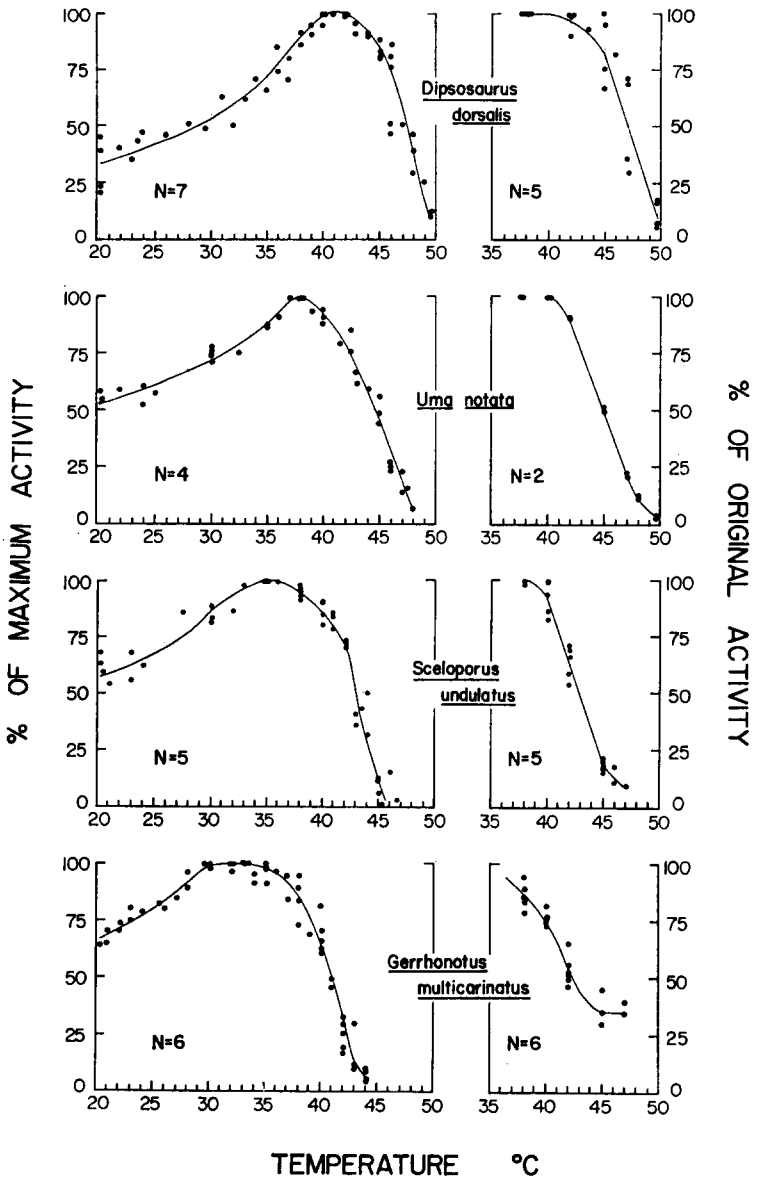

FIG. 1. Influence of temperature on activity of ATP-ase from skeletal muscle of lizards. Relative enzymic activities at various temperatures are plotted on left. The activity remaining in samples of homogenates after a 15 min incubation at various inactivation temperatures is plotted on right. N = number of individual homogenates used for each type of measurement.

optimal temperature for ATP-ase activity in each species is probably determined largely by the onset of irreversible denaturation. In all myosin preparations, except those of *Gerrhonotus*, irreversible denaturation at temperatures above the optimum parallels the decline in relative activity. Thus, the reduction in enzymic activity at these upper temperatures is probably irreversible. In the myosin from *Gerrhonotus*, the amount of denaturation decreases markedly above 42°C and there is virtually no difference between 45° and 47°C. Therefore, the reduction in activity at these temperatures is evidently at least partially reversible.

TABLE 2—THERMAL PREFERENDA AND TEMPERATURE DEPENDENCE OF ATP-ASE OF VARIOUS LIZARDS

Species	Preferred temp.* (°C)	Thermal relations of ATP-ase	
		Maximal activity (°C)	20% denatured‖ (°C)
Dipsosaurus dorsalis	38·8†	42	45·2
Uma notata	37·5‡	39	43
Amphibolurus ornatus	36·5§		44
Sceloporus undulatus	36·3‡	35	41·2
Egernia carinata	34·7§		40
Eumeces obsoletus	34·5§		39·6
Gerrhonotus multicarinatus	30·0‡	33	39·5
Gymnodactylus milii	<30§		37

* The average body temperature maintained in laboratory thermo-gradients in which all species were provided with a wide choice of temperatures.

† DeWitt, 1962.

‡ Licht, 1964.

§ Licht, Dawson and Shoemaker, unpublished.

‖ Temperature at which residual activity after 15 min incubation equalled 80 per cent of original activity.

In addition to variations in the pattern of thermal denaturation, considerable differentiation with respect to the optimal temperatures and the thermostabilities is evident among the ATP-ases from the various species (Table 2). Such differentiation is further evidenced in the comparison of the thermostabilities of the myosin ATP-ase from four additional species representing three families (Fig. 2). As in the myosin from *Gerrhonotus*, the preparations from the gecko *Gymnodactylus* are denatured at relatively low temperatures, but there is a marked reduction in the amount of denaturation at very high temperatures. It may be important that these two species are the least thermophilic of the eight studied.

Alkaline phosphatase

The relative activity of alkaline phosphatase in crude intestinal homogenates from four species of lizards is plotted against temperature in Fig. 3. Three of these

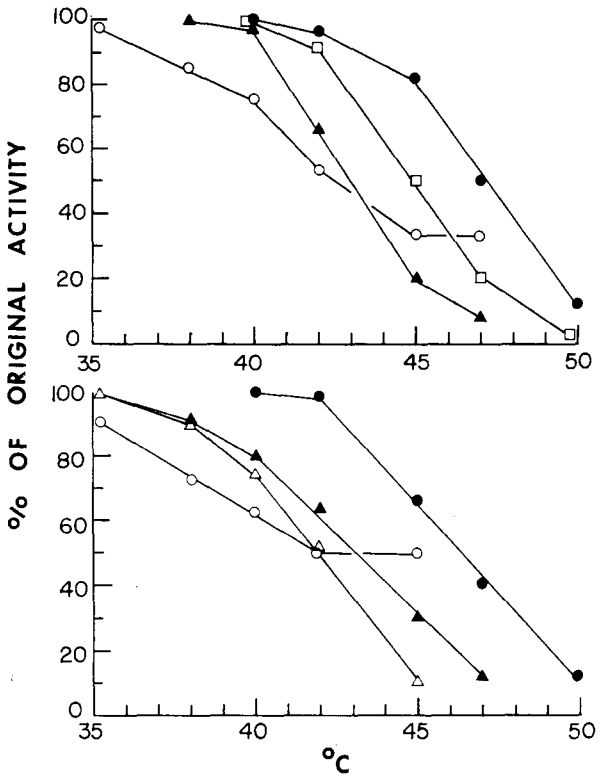

FIG. 2. Comparison of the thermostabilities of ATP-ase from several species of lizards. Curves are fitted to the mean values for each species. A (upper): ● *Dipsosaurus dorsalis*; □ *Uma notata*; ▲ *Sceloporus undulatus*; ○ *Gerrhonotus multicarinatus*. Values from Fig. 1. B (lower): ● *Amphibolurus ornatus* (3); ▲ *Egernia carinata* (3); △ *Eumeces obsoletus* (4); ○ *Gymnodactylus milii* (5). Number of homogenates of each is given in parentheses.

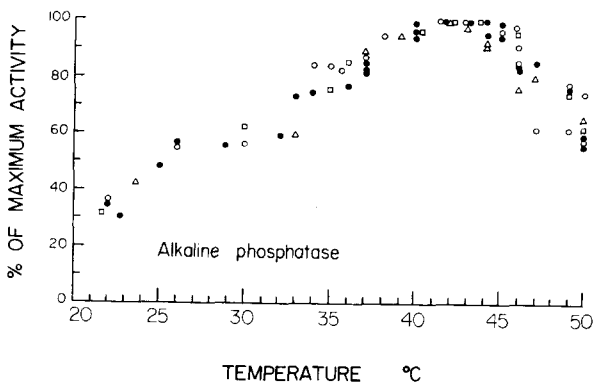

FIG. 3. Influence of temperature on intestinal alkaline phosphatase activity in four species of lizards. Number of individual homogenates used is given in parentheses: ● *Dipsosaurus dorsalis* (4); □ *Uma notata* (2); △ *Anolis carolinensis* (3); ○ *Gerrhonotus multicarinatus* (3).

species are the same as those used in the studies of ATP-ase. However, in contrast to the ATP-ase, virtually no difference in the relative activity of alkaline phosphatase at various temperatures is evident in the preparations from these species. As was observed for the ATP-ase, alkaline phosphatase activity increases with temperature up to a maximum and then decreases at higher temperatures. The maximal activity of this enzyme is obtained between 41–44°C. The slope of the curve below 30°C corresponds to a Q_{10} of 2·3, indicating an activation energy of *ca.* 14,700 cal/mole. The difference between this value and that obtained for the ATP-ase is probably not biologically significant because the alkaline phosphatase data describe an essentially linear relationship on an Arrhenius plot, whereas the values for ATP-ase do not.

With regard to thermostability, no measurable loss of activity of alkaline phosphatase from *Gerrhonotus* and *Uma* occurred during a 15 min incubation at 51°C. Thus, the decline in activity within the range of temperatures studied here is probably entirely reversible.

DISCUSSION

The present data clearly establish that there are pronounced qualitative differences in the temperature dependence of the myosin ATP-ase from various lizards. Comparison of the thermal relations of the ATP-ase with the preferred temperatures of the respective species (Table 2) indicates that both the optimal temperatures and heat resistances of the enzyme correlate well with the temperatures at which the lizards regulate (Spearman rank correlation coefficient $r_s = 0{\cdot}98$, $P < 0{\cdot}01$ for heat resistances; $r_s = 1{\cdot}0$, $P = 0{\cdot}05$ for optima). Thus, the biochemical diversity exhibited by this muscle enzyme appears to be closely related to thermal adjustments at the level of the whole animal. Presumably, these enzyme differences affect the physiological responses to temperature of the tissues in which the enzyme is operative. For example, in view of the importance of the hydrolysis of ATP in muscular contraction, interspecific variations in the thermostability of ATP-ase may underlie the differences found in the thermostability of whole skeletal muscle from various lizards (Patzl, 1933; Ushakov, 1958; Ushakov & Darevskii, 1960).

As Steinbach (1949) found in the calcium-activated ATP-ase from muscles of several temperate and tropical fishes, no significant differences are evident in the temperature coefficients of ATP-ase from lizards, i.e. adjustments to temperature are reflected primarily by a change in the position rather than in the form of the activity-temperature curve for the enzyme.

The apparent lack of qualitative differentiation in the thermal dependence of the alkaline phosphatase from lizards presents a striking contrast to the situation in the ATP-ase. The fact that the alkaline phosphatase appears to be relatively resistant to thermal denaturation in comparison with the ATP-ase suggests that it may be less important in limiting the heat tolerance of the lizards. Nonetheless, the similarity of the optimal temperatures for alkaline phosphatase activity from species which regulate body temperatures at very different levels implies that at least some lizards are operating at temperatures which are sub-optimal for their

alkaline phosphatase. Extreme individual variability in the activity of this enzyme in response to a wide array of physiological stimuli is well known (Knox *et al.*, 1956). Thus, the lizards may be able to compensate for the loss of activity incurred when living at sub-optimal temperatures by adjustments in enzyme concentration. Indeed, such seemingly adaptive changes in concentration are found in some enzymes even during short-term acclimation to temperature (Knox, 1958; Prosser, 1962).

The present data indicate that adjustments at the subcellular level may play an important role in thermal adaptation in lizards, but there are at least two distinct types of enzymes in this respect. One, such as the alkaline phosphatase from the intestine, is relatively thermostable and if adaptive modifications have occurred in this enzyme, they probably involve the level of specific activity. The second type, exemplified by myosin ATP-ase, appears to have undergone extensive shifts in temperature dependence, such that modifications in concentration are possibly but not necessarily involved. The mutability of the latter more thermolabile type of enzyme would probably be a primary requisite for adjustments to increasing temperature unless changes in enzymic concentration can be effected or alternate enzymic pathways utilized.

Acknowledgements—Acknowledgements are gratefully made to Drs. William R. Dawson and Karl F. Guthe for their guidance in the experimentation and in the preparation of this manuscript.

I am also indebted to Drs. Dugald E. S. Brown and Donald W. Misch for their helpful advice during the course of this work.

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