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Adaptations of the contractile proteins of the desert lizard *Uromastix microlepis*

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ABSTRACT

The proteins and adenosine triphosphatase activity of the contractile apparatus of *Uromastix microlepis* were studied and compared with those of a typical homoiotherm (rabbit). *Uromastix microlepis* is a diurnal lizard which inhabits the desert of the Arabian Peninsula and which experiences temperatures up to 50°C. At temperatures of 37°C, and less, the myofibrils of the lizard were less active than those of the rabbit. However, they were much more thermostable. Therefore at temperatures above 37°C the lizard myofibrils had a higher activity than the rabbit myofibrils. Energies of activation, calculated for a range of temperatures, indicated that the contractile apparatus of *Uromastix* is designed to work at relatively high physiological temperatures. Polyacrylamide gel electrophoresis suggested a possible difference in the troponin complex of the two species. The implications of enzyme systems designed to work at different temperatures are discussed.

INTRODUCTION

In recent years there have been quite a number of investigations into molecular adaptations in different animals to a wide variety of environments (see Hazel & Prosser 1974, for review). This paper briefly describes some preliminary work carried out on the characteristics of the contractile apparatus of the desert lizard *Uromastix microlepis* Blanfordi.

Uromastix microlepis is a diurnal lizard living in the desert of the Arabian Peninsula where the summer-time maximum temperature may reach 70°C. It is able to regulate its body temperature to some extent by lying in the shade, where the temperature is likely to be about 50°C. Overnight, when the temperature may drop considerably, the lizard retires to its burrow, which is usually about 1–2 m in depth. In the morning it emerges and lies in the sun for an hour or so before commencing the day's activity. During the cold winter months, when the temperature may fall to below 0°C, the lizard hibernates in its burrow, living off fat reserves (Purvis 1915). This lifestyle exposes the lizard to a wide range of temperatures but in particular it exposes it to some of the highest temperatures experienced by any poikilotherm.

Adaptations of the contractile system of poikilotherms to environmental tempera-

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tures are well documented (Johnston & Goldspink 1975; Johnston, Davison & Goldspink 1975; Johnston, Walesby, Davison & Goldspink 1975). With the possible exception of *Tilapia grahamii* from the thermal springs (40°C) of Lake Magadi, Kenya (Reite *et al.* 1974), none of the poikilotherms so far described endures temperatures much above the body temperatures of homoiotherms. It was felt therefore that a study of the myofibrillar adenosine triphosphatase enzyme of this lizard would be of considerable interest.

MATERIALS AND METHODS

Uromastix lizards between 250 mm and 500 mm long were collected from the Arabian desert in the State of Kuwait. Some of these were transported to England and kept for up to a month in the animal house in the University, Hull, where they were kept at 40°C and maintained on lettuce. Rabbits were obtained from a commercial supplier either in Kuwait or Hull.

Both the lizards and rabbits were killed by a blow to the head. Back muscles from the rabbit, and tail muscles from *Uromastix* were quickly dissected out and transferred to ice-cold 0·1 M KCl; 5 mM Tris-maleate, pH 6·5. All steps of the preparation and storage were performed at 0°C. The muscles were thoroughly homogenized on a Polytron blender and centrifuged twice at 800 × g for 15 min discarding the supernatant and resuspending in ice-cold 0·1 M KCl; 5 mM Tris-maleate, pH 6·5, to wash away catheptic enzymes. Myofibrils were then prepared according to the method of Perry & Grey (1956).

Protein concentration was estimated by the biuret reaction of Gornall *et al.* (1949) and adjusted to 5 mg. ml^{-1}.

Unless otherwise stated, adenosine triphosphatase (ATPase) assays were carried out at 20°C in 25 mM Tris-maleate, pH 7·5; 6 mM $MgCl_2$; 0·2 mM $CaCl_2$; 2·5 mM adenosine triphosphate (ATP) and adjusted to ionic strength of 0·12 with KCl. The reaction was started by the addition of ATP and magnesium, and stopped by addition of an equal volume of 10% (w/v) trichloracetic acid. The inorganic phosphate liberated was estimated according to the method of Rockstein & Herron (1951), except that the optical density was measured at 700 μm. All experiments were run in duplicate with appropriate controls and reagent blanks.

For thermal inactivation, myofibril suspension at 0°C was added to 4 volumes of the above homogenization medium at 37°C, and within 30 sec a temperature of 37°C was attained. At this time a sample was transferred to 4 volumes ice-cold incubation medium for time zero. Samples were taken at times stated and stored on ice. The sample was stirred throughout the experiment. Finally, all samples were allowed to warm to 20°C and ATPase activity was estimated.

10% acrylamide–sodium dodecyl sulphate gel slabs were prepared according to Weber & Osbourne (1969) using the Tris-buffer and 3% stacking gel described by Laemmli & Favre (1973). The samples were run on an apparatus similar to that described by Amos (1976).

RESULTS AND DISCUSSION

ATPase activities were estimated over a range of pH values (5·0–8·5) and ionic strengths (0·05–0·19) to establish optimum activity. A pH of 7·5 and an ionic strength of 0·12 were chosen and used throughout.

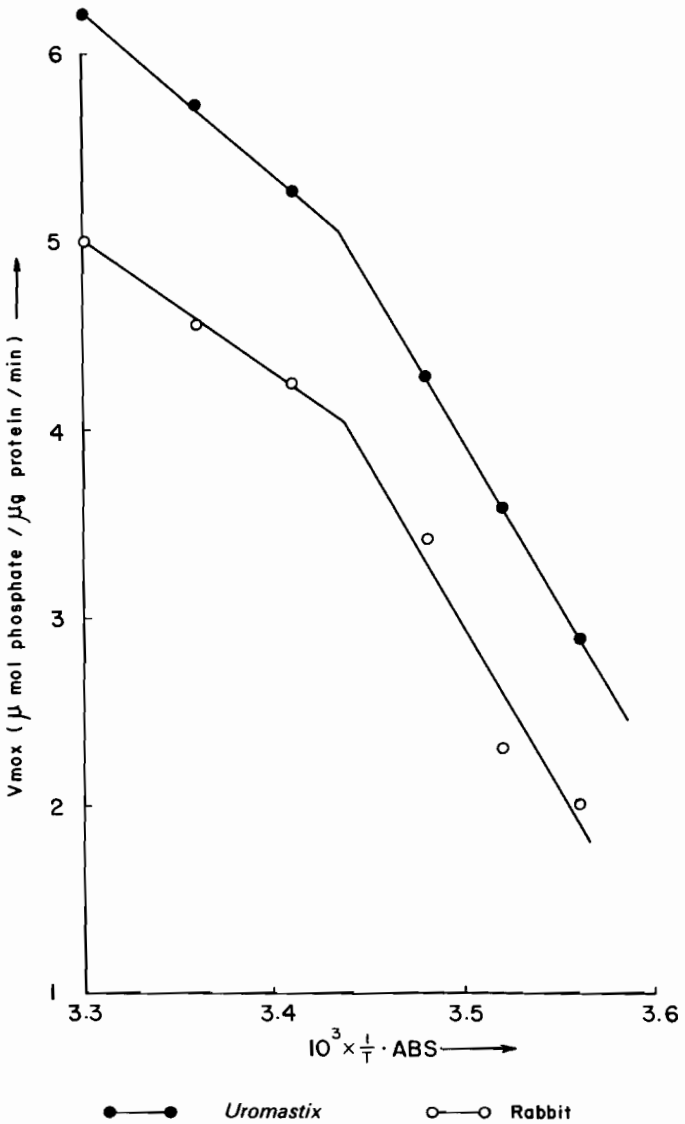

Fig. 1. Arrhenius plot of reaction velocity maximum (V_{max}, μmol phosphate liberated. μg protein^{-1}. min^{-1}) vs. 10^3 × reciprocal absolute temperature ($10^3 \times \frac{1}{T}$ · Abs.).

(a) Effect of temperature

ATPase activities were estimated over a temperature range of 8–30°C. The results are represented as an Arrhenius plot (Fig. 1). The break in the slope of the line at about 18°C has been attributed to calcium binding to the regulatory proteins (Hartshorne *et al.* 1972). From this data the energy of activation for the myofibrillar ATPase enzyme can be calculated from the slope of the straight line, which is equal to $-(Ea/R)$, (where Ea= energy of activation, cal. mol^{-1}, and R=gas constant, cal. deg^{-1}. mol^{-1}), as the specific rate for all samples had been worked out for equal amounts of protein, and is

Table 1. Energies of activation for myofibrillar ATPase enzymes (Kcal. mol^{-1})

	0°C–18°C	18°C–30°C
Uromastix	34·9	18·6
Rabbit	27·8	19·8

therefore directly proportional to the velocity constant. The results are presented in Table 1.

The values obtained for rabbit are within the range quoted by Bendall (1961) allowing for different ionic strengths.

The energy of activation is a measure of the energy to be overcome by the enzyme to proceed with a chemical reaction. The lower the value for the energy of activation the more readily the reaction proceeds. At the higher temperatures the reaction will proceed more readily for the lizard myofibrils than for the rabbit myofibrils. This would seem to indicate that this species of lizard *in vivo* had adapted to a working temperature higher than 37°C.

(b) Thermal inactivation

It can be seen from the results shown in Fig. 2 that the thermostability of *Uromastix* myofibrillar ATPase is far greater than that of rabbit at 37°C. After 30 min incubation the activity of rabbit myofibrillar ATPase had fallen by 50%, whereas the ATPase

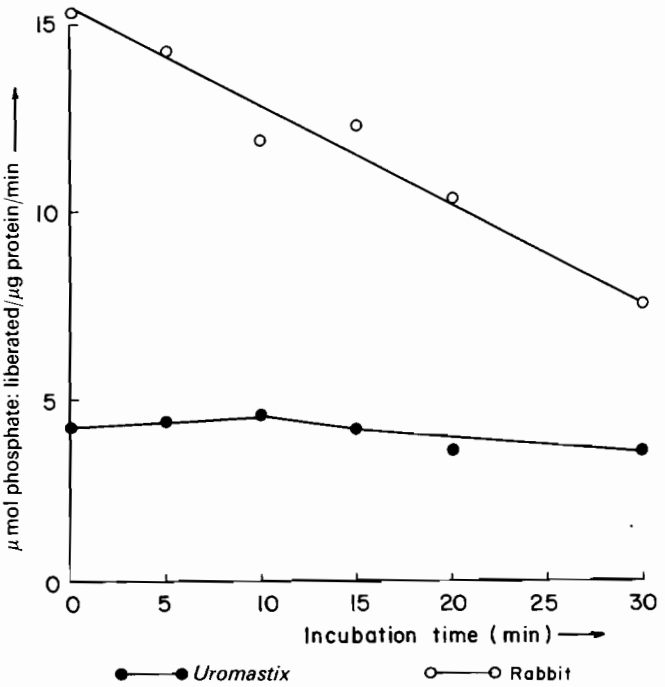

Fig. 2. V_{max} of ATPase (μmol phosphate liberated. μg $protein^{-1}$. min^{-1}) after time (min) incubation at 37°C.

Table 2. Identification of rabbit myofibrillar polypeptides on 10% polyacrylamide gel

Protein	Mol. wt.	Reference
Myosin Heavy	200,000	Lowey *et al.* (1969)
M-Line	190,000	Etlinger *et al.* (1976)
α-Actinin	180,000	Goll *et al.* (1972); Robson *et al.* (1970)
M-Line	170,000	Etlinger *et al.* (1976)
C-Protein	140,000	Offer *et al.* (1973)
Actin	47,000	Johnson *et al.* (1967); Rees & Young (1967)
Troponin-T	37,000	Wilkinson *et al.* (1972)
Tropomyosin	35,000	Wilkinson *et al.* (1972); Weber & Osbourne (1969)
Light Chain 1	25,000	Lowey & Risby (1971)
Troponin-I	24,000	Wilkinson *et al.* (1972)
Troponin-C	20,000	Wilkinson *et al.* (1972)
Light Chain 2	18,000	Lowey & Risby (1971)

activity of *Uromastix* had fallen by only 20%. Thus *Uromastix* myofibrils are far more thermostable than those of the rabbit. This is unusual as the findings of Arai *et al.* (1976) indicate that myofibrils of most poikilotherms are much less resistant to thermal denaturation than rabbit myofibrils. This again suggests that the *Uromastix* lizard is adapted to function at temperatures higher than the body temperature of homoiotherms.

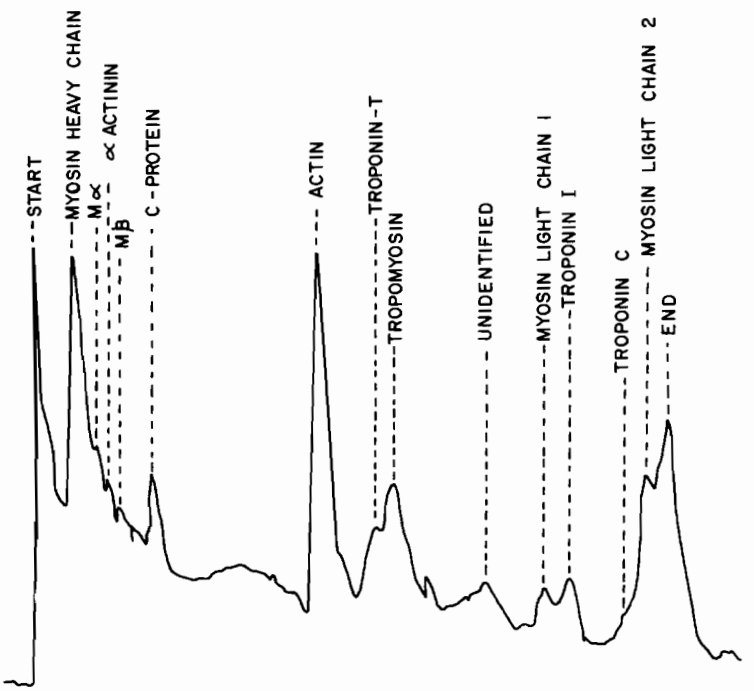

Fig. 3. Microdensitometric scan of polyacrylamide gel slab showing rabbit myofibrillar polypeptides. Molecular weights derived from Table 3.

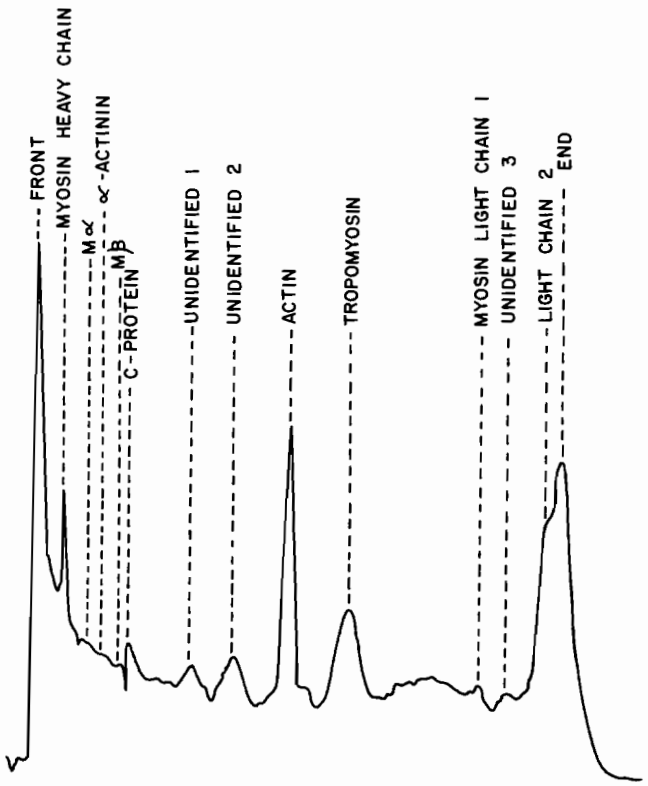

Fig. 4. Microdensitometric scan of polyacrylamide gel slab showing *Uromastix microlepis* myofibrillar polypeptides. Molecular weights of unidentified polypeptides are shown in Table 3.

(c) Polyacrylamide gel electrophoresis

The sodium dodecyl sulphate–polyacrylamide gel electrophoresis pattern for rabbit myofibrillar polypeptides has been thoroughly worked out by previous workers (Table 2). This system has been used to calibrate the slab to identify the polypeptides of *Uromastix*. Microdensitometric scans of the runs are presented for the rabbit (Fig. 3) and *Uromastix* (Fig. 4).

The components of myosin, actin and tropomyosin appear to be common between rabbit and *Uromastix*. However, the troponin complex in *Uromastix* appears to either have been lost or, more probably, altered in molecular weight as it does not occupy the usual position on the gel slab. It may indeed be with the unidentified proteins 1, 2, & 3

Table 3. Approximate molecular weights of unidentified *Uromastix* polypeptides

Number	Molecular Weight
1	89,000
2	68,000
3	21,500

(Table 3). It is possible that the regulatory proteins play a major part in thermal adaptation to the environment in the contractile apparatus. However this aspect needs further investigation.

It is often stated in biology textbooks that as the body temperatures of homoiotherms represent optimal temperatures for the activity of enzymes, higher temperatures will lead to denaturation. This work shows that in the *Uromastix* lizard, which functions at higher temperatures than 37°C, the main enzyme of the contractile system has been modified accordingly. The authors wish to suggest that 37°C (40°C in birds) has been adopted as the body temperature because this, in most cases, allows the animal to lose heat to the environment during and immediately following activity. It does not seem to be a question of optimum enzyme activity, as enzyme systems have been designed which will function at different temperatures, ranging from -1°C to $+2$°C for Antarctic fish (Johnston, Walesby, Davison & Goldspink 1975) to up to 50°C for the desert lizard.

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تكيف البروتينات المنقبضة في الضب الصحراوى
يوروماستكس مايكروليبيس

روجر بنى و جيفرى جولدسبنك

وحدة بحوث العضلات ، قسم علم الحيوان بجامعة هل ، المملكة المتحدة

خلاصة

قام الباحثان بدراسة نشاط البروتينات وانزيم الاتيبيز في عضلات الضب يوروماستكس مايكروليبيس ومقارنته بنظيره في الارنب ، وركز الباحثان في هذه الدراسة على اثر الحرارة في نشاط البروتينات . وقد وجدا ان نشاط الالياف العضلية الدقيقة في الضب اقل منه في الارنب عند درجة حرارة ٣٧° م وما دونها ، اما فوق هذه الدرجة فان نشاط الالياف في الضب اعلى منه في الارنب ، ويعزى ذلك الى ان الياف الضب اكثر ثباتا عند درجات الحرارة العالية .

كما وجد الباحثان ، عند حسابهما لطاقات تشغيل عضلات الضب في درجات حرارة مختلفة ، ان هذه العضلات مصممة للعمل تحت درجات حرارة مرتفعة . وباستخدام عملية الفصل الكهربى في وجود البولى اكريل اميد وجد ان هناك اختلافا بين مركب التروبونين في الارنب ومثيله في الضب .