ENZYME (ALDOLASE) ACTIVITY IN HYPEROSMOTIC MEDIA (NaCl AND UREA) 
IN THE TERRESTRIAL TOAD, BUFO VIRIDIS AND FROG RANA RIDIBUNDA

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ABSTRACT

In this study we examined the adaptation of the enzyme Aldolase from frogs and toads to different temperatures and to hyperosmotic media of 400-1000mOsm/Kg of urea or NaCl. Maximum enzyme activity was found between 200-400mOsm/Kg NaCl, both in enzymes from green toads and from marsh frogs. However, above 500mOsm/Kg, the activity of enzymes from green toads was significantly higher than the activity of enzymes from marsh frogs.

The activity of aldolase from green toads decreased very slowly as the media concentration of urea increased. However, the activity of aldolase from marsh frogs decreased rapidly under the same conditions.

The maximum activity of aldolase from both frogs and toads was at 25°C. The activity of aldolase from green toads was significantly higher than the activity of aldolase from marsh frogs when measured only at high temperatures (35°C). The results of this study support the idea that the biochemical systems of terrestrial amphibia are tolerant to hyperosmotic media.
INTRODUCTION

The green toad is terrestrial and lives in a wide range of habitats reaching the southern limit of its distribution in Israel, where it penetrates into the desert (Warburg, 1972). Katz (1973) succeeded in adapting green toads to 800mOsm/Kg NaCl and Degani and Hahamou (1985b) adapted the green toad to 800mOsm/Kg NaCl. Degani et al. (1984) found that after three months in the soil, blood plasma concentration reached a maximum of 1400mOsm/Kg with urea concentration at 900mM. Intracellular urea concentration in the muscle of these toads amounted to 500mM urea. The plasma osmolarity of R. ridibunda did not reach 600mOsm/Kg in any of these conditions (Katz, 1973; Degani, 1985a). R. viridis appears to show an ability to tolerate a wide variety of osmotic plasma concentrations, which help it to adapt to a wide variety of osmotic environments.

Urea accumulation in the intra — and — extracellular fluids appear to play a critical role in adaptation of amphibians to terrestrial life (Bentley, 1966; McClanahan, 1967 and 1972; John, 1910, 1923; Degani et al., 1981, 1984). Data presented by McClanahan (1972) and Jones (1982) suggested that the rate of urea synthesis increases in Xenopus laevis, Scaphiopus couchi, Bufo woodhousei and Hyla cinerea, when the water potential of the environment decreases. During dehydration, the plasma, urine and intracellular body fluid osmolalities of these amphibians were elevated. This was caused not only by increased urea but also by increased concentrations of Na+, Cl- and K+ (Degani and Warburg, 1984). However, there is no information on the adaptation of enzymes to such high concentrations of urea and NaCl.

The hypothesis examined in this study is whether the enzymes of terrestrial amphibia are more tolerant to high temperatures, and to urea and NaCl concentrations, than aquatic species. In the present study the activity of Aldolase was compared in the green toad (Bufo viridis) and the semi-aquatic marsh frog (Rana ridibunda).

MATERIAL AND METHODS

Green toads (R. viridis) and marsh frogs (R. ridibunda) were collected from the same locality in Northern Israel (Naharia) and were kept in the laboratory in a terrarium containing soil and small vessels of water, at a temperature of 20-23°C. Samples of muscle were taken (200mg) from the hind-legs of green toads and marsh frogs and the fructose — 1.6 Diphosphate Aldolase activity was determined after homogenization (polytron homogenizer, model Kinematic CH-5010 Krimi-LU-Switzerland) in 1ml TEM buffer phosphate pH 7.8.

The assay of activity was performed as described by Gray et al. (1970). Aldolase activity was measured after the enzymes were incubated for 20 minutes in different solutions of NaCl (from 100 to 1000mOsm/Kg) or urea (from 400 to 1000mOsm/Kg). Enzyme activity was also measured at different temperatures (15°C, 20°C, 25°C, 30°C and 35°C) in a solution of 300mOsm/Kg. Aldolase activity was measured by monitoring the rate of oxidation of NADH at 340nm on a Gilford Spectrophotometer at 25°C. One unit of enzyme activity is defined as (µmol of substrate (FDP) that is cleaved in one minute at 25°C. Aldolase activity was calculated to nM by the equation: 100D = 0.0089µmole/min/mM = 0.0089 unit of activity (U).

RESULTS

The activity (%) of aldolase during acclimation to NaCl solution up to 1000mOsm/kg is shown in Fig. 1. Maximum enzyme activity was found between 200-400mOsm/Kg NaCl, both in enzymes from green toads and from marsh frogs. However, above 500mOsm/Kg NaCl, the activity of enzyme from green toads was significantly higher than the enzyme activity of marsh frogs.

![Fig. 1 Comparison between enzyme activity of Bufo viridis and Rana ridibunda in different NaCl osmotic pressures.](image)

Fig. 1

There were no significant differences found between the aldolase activity from green toads or marsh frogs acclimated to 400mOsm/Kg (200mOsm/Kg NaCl and 200mOsm/Kg urea) (Fig. 2). In media of osmolarity above 400mOsm/Kg NaCl increase due to additional urea caused the activity of aldolase from R. viridis to be higher than that of the enzyme from R. ridibunda. The activity of aldolase from marsh frogs decreased rapidly after acclimatization to a high urea concentration, and the activity of aldolase from green toads decreased slowly (Fig. 2). Both enzymes from green toads and from marsh frogs decreased with a linear correlation to increasing media urea concentration (Table 1). The slope of the linear equation for green toads is smaller than the slope of the linear equation for marsh frogs.

The maximum activity of aldolase from both green toads and marsh frogs was found at 25°C (Fig. 3). There was no significant difference between the activity of aldolase from green toads and marsh frogs at temperatures of 15-30°C. However, there is a significant difference only at a temperature of 35°C (P<0.05; t-test). At this temperature the activity of aldolase from green toads was the same as the activity of aldolase from marsh frogs.
**ENZYME ADAPTATIONS IN ANURANS**

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**Fig. 2** Enzyme activity from *Bufo viridis* and *Rana ridibunda* in different ionic solutions.

**Fig. 3** Temperature effect on enzyme activity from *Bufo viridis* and *Rana ridibunda*.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Rana</th>
<th></th>
<th></th>
<th>Equation</th>
<th>Bufo</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>$y = -0.8069x +1345$</td>
<td>0.95</td>
<td>11</td>
<td>&lt;0.001</td>
<td>$y = -0.5430x +1259$</td>
<td>0.87</td>
<td>13</td>
</tr>
<tr>
<td>Urea</td>
<td>$y = -1.7109x +2170$</td>
<td>0.91</td>
<td>22</td>
<td>&lt;0.001</td>
<td>$y = -0.3946x +1523$</td>
<td>0.71</td>
<td>12</td>
</tr>
</tbody>
</table>

**TABLE 1:** Enzyme activity equations of *Bufo viridis* and *Rana ridibunda* acclimated to different solutions according to the most significant correlation.

**Notations:**
- $Y =$ Enzyme activity (mm) and $X =$ osmolality (mOsm/kg).
- There are significant differences ($P < 0.05$) between the slopes of both pairs Rana : Bufo in NaCl, Rana : Bufo in Urea.

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**DISCUSSION**

The osmotic concentration recorded in the body fluid of green toads following prolonged dehydration in the soil is the highest reported so far for any terrestrial amphibian (Degani *et al.*, 1984). A similar high osmotic concentration was recorded in the terrestrial spade-foot toad (*Scaphiopus couchii*) (Shoemaker, 1969; McClunahan, 1972) from the Arizona desert. Both *Bufo* and *Scaphiopus* store urea in their body fluids and this allows them to raise the bodily osmotic pressure more efficiently than aquatic species (Balinsky, 1981; Degani *et al.*, 1984).

There are some physiological mechanisms described which increased the plasma osmolality by increasing the plasma Na⁺, Cl⁻ and urea during dehydration or saline adaptation. For example by reducing the glomerular filtration rate of the nephron and increasing the influx of Na⁺, Cl⁻ through the nephron, bladder and the skin (Jorgensen, 1950; Bentley, 1958; Maetz, 1968). Accumulation of urea occurs slowly and may be due to both urea retention and, possibly to increased synthesis due to a higher concentration of pressorins in the urea cycle (Jones, 1980; Balinsky, 1981; Lee *et al.*, 1982).

In this study we found that aldolase activity is tolerant to a high urea concentration only in terrestrial green toads but not in semi-aquatic marsh frogs. These results might explain the results of previous studies in our laboratory. The accumulation of urea in the plasma of green toad is accompanied by an intracellular space both in vivo and in vitro (Degani *et al.*, 1984; Degani, 1985a, b). In the green toad from soil the intracellular urea is less concentrated than that in the plasma. (Degani *et al.*, 1984). The urea concentration in the plasma of the green toad was significantly higher than that in the plasma of the marsh frog, both adapted to a hypotonic (urea) environment (Degani, 1985a). Crab-eating frogs (*Rana cancrivora*), the only anuran that lives in seawater had a maximal urea concentration in the plasma of 350mM (Gordon *et al.*, 1961) and this is similar to the urea concentration (265mM) found in the plasma of green toads adapted to seawater (Katz, 1973). The muscle of the crab-eating frog is not only tolerant to urea, but actually requires it. Thiele and Schmidt-Nielsen (1962) were unable to observe contractions unless urea was present in the medium, and they routinely added 250mOsm urea to the medium bathing the muscle.

Muscle tissue of green toads seems more tolerant to high urea concentration than the muscle of marsh frogs (Degani, 1985a). In conclusion while these previous studies show that different physiological mechanisms are tolerant to high urea concentrations,
this study shows that in terrestrial amphibians there might also be enzymes which are able to adapt activity to these conditions (Dechon and Whitford, 1973; Licht et al., 1975; Degani, 1983a). Rapid dehydration or adaptation to salinity causes an elevation of Na⁺ and Cl⁻ in the tissues (Degani and Warburg, 1984) so that the enzymes are exposed to high Na⁺ and Cl⁻ concentrations (Degani, 1985a). In this we found that an enzyme (aldolase) of green toads tolerates a higher concentration of Na⁺ and Cl⁻ than does the same enzyme from marsh frogs and this is maybe a partial explanation of the greater tolerance both to dehydration and to saline environments of the toad compared to the frog.

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