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Kinetic Behaviour of AMP Deaminase with Reference to *p*H and Temperature in the Gastrocnemius Muscle of *Rana hexadactyla* during Sciatectomy

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The activity of AMP deaminase was increased significantly in the neurectomized gastrocnemius muscle of frog. An optimum *p*H of 7.0 was observed for both the contralateral and denervated muscle enzymes. The *p*H versus temperature relationship studies showed increased activity of the denervated muscle enzyme as compared to the contralateral muscle enzyme. The activation energy values (Δ E) were found to be higher for contralateral muscle enzyme. The V_{max} values gradually increased up to 7.0 *p*H and from there declined in case of both the contralateral and denervated muscle enzymes. The *p*H versus $-\log K_m$ (pK_m) relationship showed the involvement of imidazolyl groups of histidine and amino groups of cysteine in the catalysis.

Key Words: *Rana hexadactyla*, **AMP deaminase, Sciatectomy, Activation energy, Maximal velocity, Michaelis-Menten constant**

Introduction

It has been suggested by the earlier workers that the ammonia formation of working muscle is due to the activity of AMP deaminase, a member of purine nucleotide cycle. (Embden et al. 1928, Needham 1932, Lowenstein 1972.) Kornberg (1957), Buchanan and Hartman (1959) have reported that the nucleotide can be formed (*de novo*) by the so-called salvage pathways starting from preformed bases or nucleosides. The mechanism of regulation of this enzyme is by deamination of AMP (ATP activated) followed by dephosphorylation of IMP (direct pathway) or dephosphorylation of AMP (ATP inhibited) followed by deamination of adenosine (indirect pathway). Besides widely distributed in animal tissues, AMP deaminase is predominantly an extramitochondrial, non-particulate enzyme and its metabolic role is supposed to be important in amino acid degradation, energy stabilization and ammonia production

(Lowenstein 1972). Several conflicting reports are available regarding its activity in dystrophic and atrophic muscles of various animals (Pennington 1962) Heyck et al. 1963, Turner & Manchester 1972). The denervation studies have considerable neurochemical importance, because they give information on various less known aspects of the complex inter-relationships between nerve and tissue cells (Gutmann 1973). The denervation of skeletal muscle causes marked atrophied condition which results in the alteration of the biochemical and physiological properties of the muscle (Close 1972). The AMP deaminase activity was studied thoroughly in mammals by several investigators (Pennington 1962, Manchester 1972) and scant attention was paid to the non-mammals especially amphibians. Hence in the present study an attempt has been made to study the *p*H and temperature effects on kinetic behaviour of this enzyme in the denervated gastrocnemius muscle of frog, *Rana hexadactyla* (Lesson).

Material and Methods

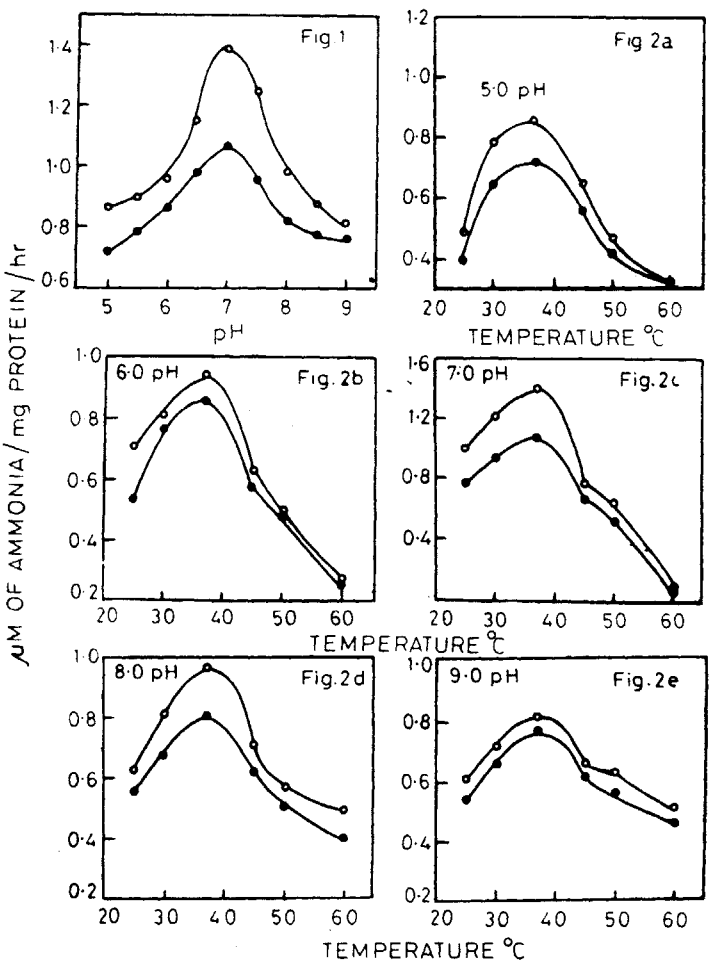
Rana hexadactyla (Lesson) of healthy and medium size were denervated by sciatic nerve section about 1 cm from its origin in one side of the leg, while the contralateral muscle was considered as control. The frogs were fed *ad libitum* with cockroaches, and water was changed regularly. After 30 days, post-operatively, animals were sacrificed and both the denervated and contralateral gastrocnemius muscles were excised quickly. Homogenates of tissues (5% wt/vol) were prepared in ice-cold double distilled water, using Potter-Elvehjem type glass homogeniser and centrifuged at 2,500 rpm for 15 min to remove the cell debris, and the supernatants were dialysed overnight in a dialysis bag at 0°C against suitable medium (double distilled water). The AMP deaminase (EC 3.5.4.6) activity was estimated by the method of Weil-Malherbe and Green (1955) with slight modifications, considering the amount of free ammonia liberated from the AMP (substrate) (Wagelin et al. 1978). The enzyme activity is expressed as μm of ammonia formed/mg protein/hr. The maximal velocities (V_{max}), and Michaelis-Menten constants (K_m) were determined from Lineweaver Burk plots. The activation energy (ΔE) values were calculated as given by Dixon and Webb (1964). The protein content was estimated by the method of Lowry et al. (1951). All substrate saturation curves were plotted with the average values of six observations at individual substrate concentrations to minimize the deviation.

Results and Discussion

Variation in the *p*H are known to alter the metabolic activity due to changes in the enzyme activity. However, enzymatic studies are generally conducted at the optimum *p*H, where the enzyme expresses its maximal activity (Burch et al. 1963, Frieden 1963). In the present study the enzyme showed a *p*H optimum centered at *p*H 7.0. The dependency of the enzyme activity of both the contralateral and denervated muscles showed similar pattern (figure 1). Pezzini et al. (1975) have reported an optimum *p*H of 7.2 for AMP deaminase from rat skeletal muscle. An optimum *p*H of 6.68 and 6.5 were reported for AMP deaminase of rabbit and human skeletal muscles (Lee 1957, Makarewicz & Stankiewicz 1974).

The comparison of peak activities at different *p*H and temperatures showed a

general decrement in the peak activity level with increase in temperature (figure 3), but when compared to the contralateral control an elevated modulation of activity in the denervated muscle enzyme by both temperature and *p*H was observed (figure 2). In alkaline *p*H range at 60°C the enzyme was still showing some activity in both the contralateral and denervated muscles suggesting that AMP deaminase is less sensitive to the thermal denaturation which is well in line with the findings of Makarewicz and Stankiewicz (1975) and Kaletha (1976) who have reported that AMP deaminase is less susceptible to thermal denaturation. At optimum temperature (37°C) the differences in the activity levels between contralateral and denervated muscle enzymes at various *p*H values were more or less the same suggesting that the groups involved in the catalysis were unaffected. This shows that a sort of protection for the enzyme



Figures 1–2 *1*, *p*H profile curves of AMP deaminase of the contralateral and denervated muscle; *2*, Thermal modulation of AMP deaminase of the contralateral and denervated muscles at different *p*H values

observed in the denervated muscle may be due to some of the altered cytosolic components (presumably proteins) which may not involve the primary catalytic site (Radhakrishna Murthy 1974).

The log V_{max} vs. 1/T or Arrhenius plots (figure 5) showed that the curves are not linear as expected for a heterogeneous catalyst like enzyme (Lumry 1959, Dixon & Webb 1964), suggesting there are temperature-dependent changes in the activation energy. The catalytic efficiency is often measured in terms of the decrease in the activation energy and lower the activation enery, higher the efficiency and *vice versa*. The activation energy depends on the ionization of the catalytic groups, which in turn depends on the *p*H of the medium (Radhakrishna Murthy 1974).

However, in general, the contralateral muscle homogenate enzyme had increased Δ E values as compared to the denervated muscle homogenate at all *p*H ranges studied. But, the extent of increase was found to be more in the acid range as compared to the alkaline range. This suggests that the enzyme may be more efficient in alkaline ranges rather than in acid ranges. A change in the *p*H by one unit decreased the Δ E values to a considerable extent in both the contralateral and denervated muscle homogenates. However, there is a gradual fall in these values as the *p*H shifts to more alkaline ranges. In evidence to this, Eyring and Stearn (1939) have recorded changes in Δ E values for

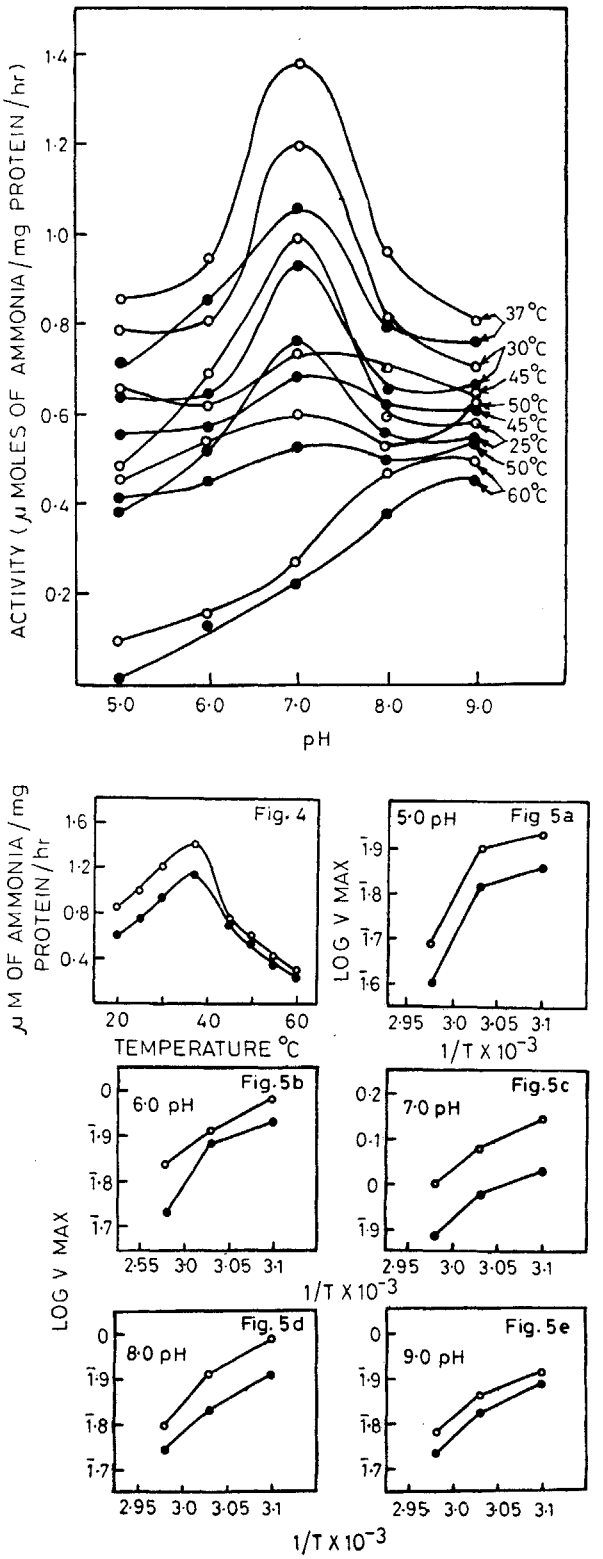


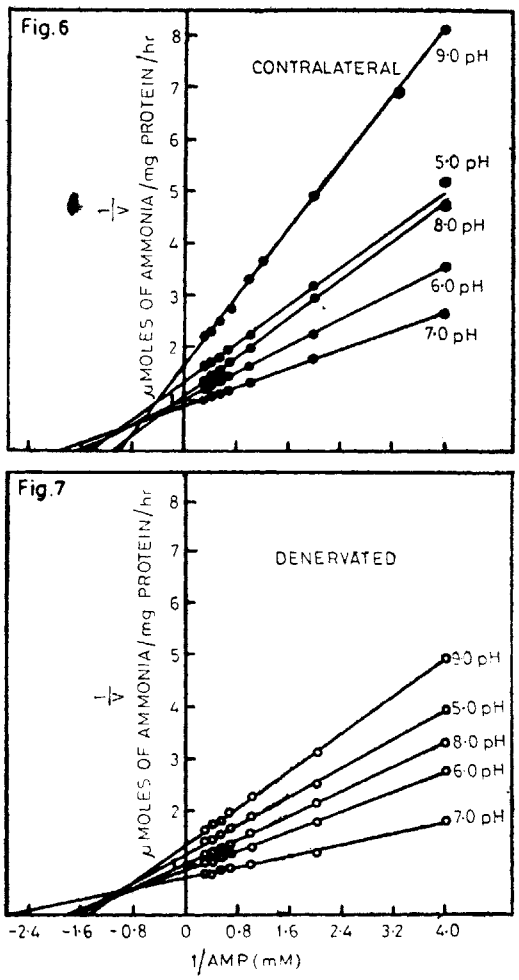
Figure 3 *p*H profiles at different temperatures for both the contralateral and denervated muscle AMP deaminases

Figures 4–5. *4*, Temperature effects on AMP deaminase activity of the contralateral and denervated muscles, *5*, Arrhenius plots at different *p*H values for AMP deaminase of the contralateral and denervated muscles

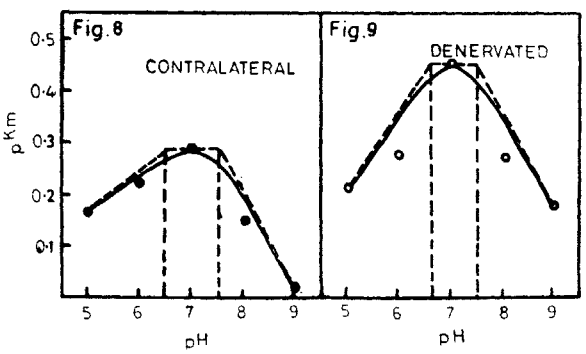
the denaturation of egg albumin in water at 65°C with *p*H changes.

Lineweaver-Burk plots (figures 6 & 7) showed that the V_{max} values were gradually increased up to 7 *p*H and from there declined in case of both the contralateral and denervated muscle enzymes. Whereas the slope, intercept and K_m values decreased from 5.0 to 7 *p*H and then showed increase in both the cases (table 1). The elevated activity of AMP deaminase in the denervated muscle consequent to neurectomy can be more clearly envisaged from the decreased K_m and increased V_{max} values as compared to the contralateral muscle enzyme at all *p*H ranges. The lesser the K_m value, the higher is the affinity of the enzyme for substrate and *vice versa*. The elevated activity of AMP deaminase in the denervated muscle, therefore, is related to an increase in the active site density of the enzyme and also its affinity for AMP.

*p*H versus –log K_m (pK_m) relationships (figures 8 & 9) were plotted to determine the ionisable groups. The amino acid chains were found to have imidazolyl groups of histidine (5.6–7.0 *p*H) and amino groups of cysteine (6.5–7.5) (Dixon & Webb 1964). Boosman and Chilsong (1976) studied the amino acid composition of AMP deaminase from chicken and rabbit skeletal muscle and reported the presence of 18 amino acids having histidine and cysteine. The denervation process did not affect the active site ionization pattern in general, but there was a difference in the degree of slopes and inflections at 6.5–7.5 *p*H, which might be responsible for altered enzyme activity in the denervated muscle.



Figures 6–7 Double reciprocal plots of substrate and activities of contralateral and denervated muscle AMP deaminases at different *p*H values



Figures 8–9 Plots of-log Michaelis-Menten constants (pK_m) as a function of *p*H of AMP deaminase activity of the contralateral and denervated muscles

•——• Contralatreal, o——o Denervated

Table 1 *Kinetic parameters of AMP deaminase at different pH values. (V_{max} values are represented as μmoles of ammonia formed/mg protein/hr)*

pH	Contralateral muscle homogenate				Denervated muscle homogenate			
	Slope	Intercept	K_m (mM)	V_{max}	Slope	Intercept	K_m (mM)	V_{max}
5.0	0.9041	1.3514	0.669	0.74	0.7227	1.2005	0.602	0.833
6.0	0.6026	0.988	0.6098	1.012	0.467	0.8601	0.5435	1.1627
7.0	0.4773	0.935	0.5102	1.069	0.2455	0.7168	0.3425	1.395
8.0	0.7762	1.1025	0.704	0.907	0.5904	0.9718	0.6075	1.029
9.0	1.604	1.7007	0.9434	0.588	0.8696	1.3399	0.649	0.7463

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