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CATALYTIC POTENTIAL OF GLUTAMATE DEHYDROGENASE IN NORMAL AND FATIGUED GASTROCNEMIUS MUSCLES OF FROG

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ABSTRACT

Substrate dependent and co-factor dependent kinetic parameters of glutamate dehydrogenase (GDH) have been investigated in the normal and fatigued gastrocnemius muscles of frog (*Rana hexadactyla*). The fatigue phenomenon seems to induce a mixed type of inhibition by decreasing the maximal velocity (V_{max}) and increasing the Michaelis-Menten constant (Km). The activation energy values for the muscle enzyme were increased on fatigue, suggesting a decreased catalytic efficiency of fatigued muscle enzyme.

INTRODUCTION

WHEN a gastrocnemius muscle is subjected to repeated electrical stimuli, fatigue substances accumulate in the tissue and inactivate the enzyme systems[1,2], inducing fatigue[3]. A significant decrease in succinate, malate, lactate dehydrogenases and pyruvate oxidase[4] and an increase in protease activity levels[5] have been reported during muscular fatigue. This increased proteolysis may affect the existing ammonia metabolism in the tissue. Since GDH is known to play a significant role in the regulation of ammonia metabolism of the tissue, an attempt has been made in the present investigation to study the kinetic parameters of the enzyme so as to assess the specific impact of fatigue on the catalytic potential of the enzyme.

MATERIALS AND METHODS

Healthy medium sized frogs, *Rana hexadactyla*, were double pithed and the gastrocnemius muscles from both the legs were excised with least injury. The muscles were washed 3 to 4 times in amphibian Ringer's medium[6] and then allowed to stand in the same solution for ten minutes to recover from shock effects. One of the muscles was placed in 10 ml of amphibian Ringer's medium and subjected to repeated biphasic direct electrical stimuli of 10 volts at a pulse frequency of 60/min using Inco/CSIO research stimulator Model MR (Ambala-3, India). The stimuli were given continuously until the muscle did not respond to fresh stimuli, exhibiting fatigue phenomenon. The contralateral muscle was also kept in the similar medium and was not subjected to electrical stimulation. Both these muscles were chilled to 5° C to arrest residual metabolism and 10% (W/V) homogenates were prepared separately in 0·25 M sucrose solution using Potter-Elvehjem homogenizer and centrifuged at 2500 rpm for 15 min to remove the cell debris and the supernatants were dialyzed overnight in a dialysis bag at 0° C against the suitable medium (0·25 M sucrose solution). The GDH (EC 1.4.1.3) activity was estimated by the method of Lee and Lardy[7] modified by Pramilamma *et al.*[8] and the enzyme activity is expressed in μmoles of formazan/mg protein/hr. The maximal velocities (V_{max}), Michaelis-Menten constants (Km) were calculated by the method of least squares. The activation energy (E) was calculated as given by Dixon and Webb[9].

RESULTS AND DISCUSSION

After initial standardization, the activity levels of NAD-GDH were determined in normal and fatigued gastrocnemius muscle homogenates of frog. An enzyme concentration of 50 mg and 30 minutes of incubation time were selected for the present study to ensure initial velocity. The substrate dependent activity of GDH was studied at 7·4 pH (sodium phosphate buffer) and 37° C (optimum temperature) with 0·1 mM concentration of NAD (co-factor) and with graded substrate concentrations ranging from 0·5 to 15 mM of glutamate.

Substrate concentrations *versus* velocity plots for GDH of normal and fatigued muscles have revealed that the enzyme activity was linear with the substrate concentration of 5 mM and at concentrations above 5 mM the reaction entered zero order zone suggesting the abolition of substrate dependency indicating saturation of the enzyme at 5 mM of substrate. Line Weaver-burk plots for the activities of GDH of normal and fatigued muscles were drawn with the reciprocal substrate concentrations and the activity levels showed a variation in the kinetic parameters like maximal velocity (V_{max}) and Michaelis-Menten constant (Km) (Table I, Fig. 1).

TABLE I

Kinetic parameters of GDH activity at 7·4 pH in normal and fatigued muscles of frog

(V_{max} values are expressed in μmoles of formazan/mg protein/hr)

Enzyme source	Glutamate (substrate)		NAD (co-factor)	
	V_{max}	Km (mM)	V_{max}	Kp (mM)
Normal muscle	0·179	1·098	0·205	0·009
Fatigued muscle	0·147	1·333	0·141	0·011
% deviation over normal muscle	−17·9	+21·4	−31·2	+22·2

Fig. 1

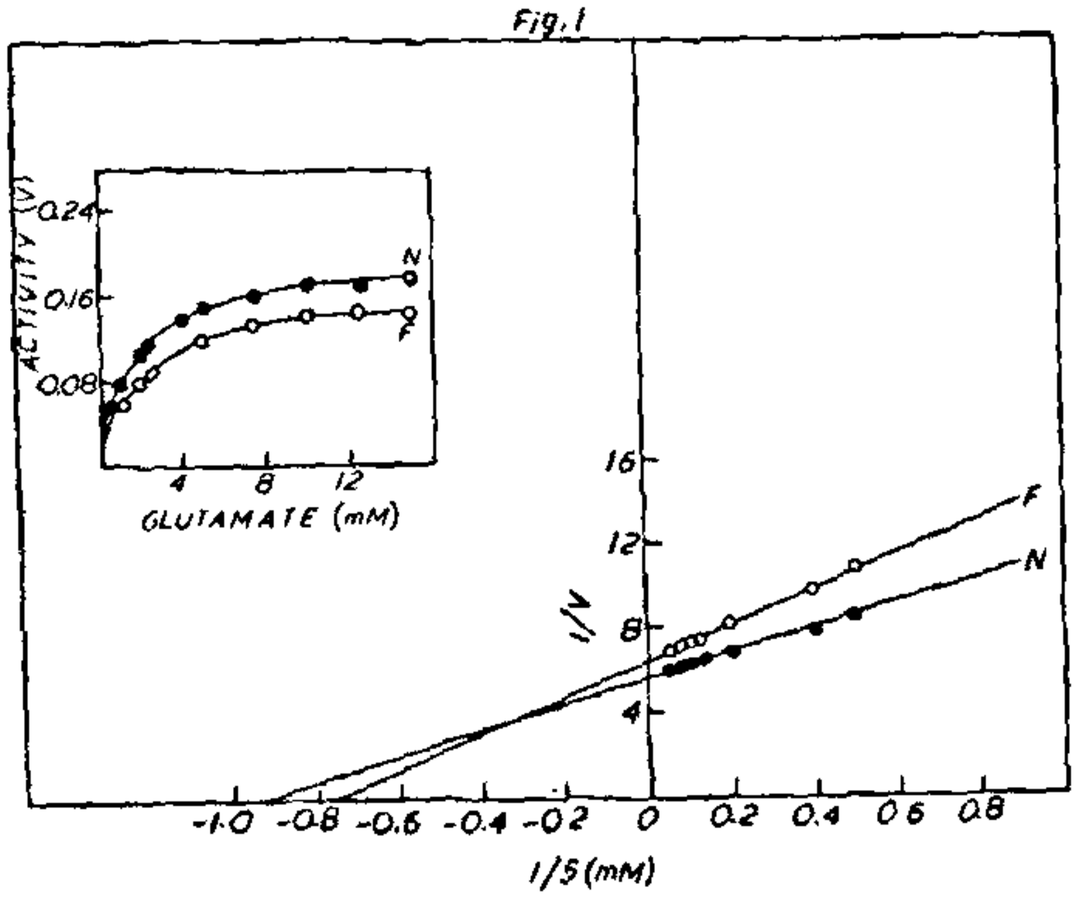

FIG. 1. Double reciprocal plots of substrate (glutamate) *versus* activities of glutamate dehydrogenase of the normal (N) and fatigued (F) muscles at 7·4 pH along with the substrate *versus* activity curves.

V_{max} values were found to be 0·179 and 0·147 μmole of formazan/mg protein/hr for the normal and fatigued muscle enzymes respectively showing 17·9% decrement, suggesting significant masking of active sites during fatugue. The decreased enzyme activity during fatigue may be due to augmented proteolysis[5, 10] which could bring about diminished catalytic activity, due to the accumulation of products of partial oxidation of carbohydrates, fats and proteins such as lactic acid, oxaloactate, hydroxybutyrate and acetate which alter the ionization pattern of the enzyme molecule resulting in the enzyme inactivation[11]. Increased production of lactic acid[12–15] may bring about the mitochondrial swelling[16] as muscle mitochondria are amenable to changes in structure and function under metabolic stress[17, 18] resulting in catalytic impairment of this enzyme. Ammonia levels were found to increase in the earlier stages of contraction[19, 20] which might cause product inhibition on the enzyme[21,22]. The Km values were increased from 1·098 to 1·333 on fatigue indicating reduction in the ES complex formation due to the decreased enzyme substrate affinity. Thus it appears that fatigue phenomenon may be imposing a sort of mixed type of inhibition on the GDH enzyme by altering both V_{max} and Km and affecting the overall catalytic efficiency of the enzyme.

The co-factor (NAD) *versus* velocity relationship also showed a similar trend during the onset of fatigue (Fig. 2). The V_{max} showed 31·2% decrement

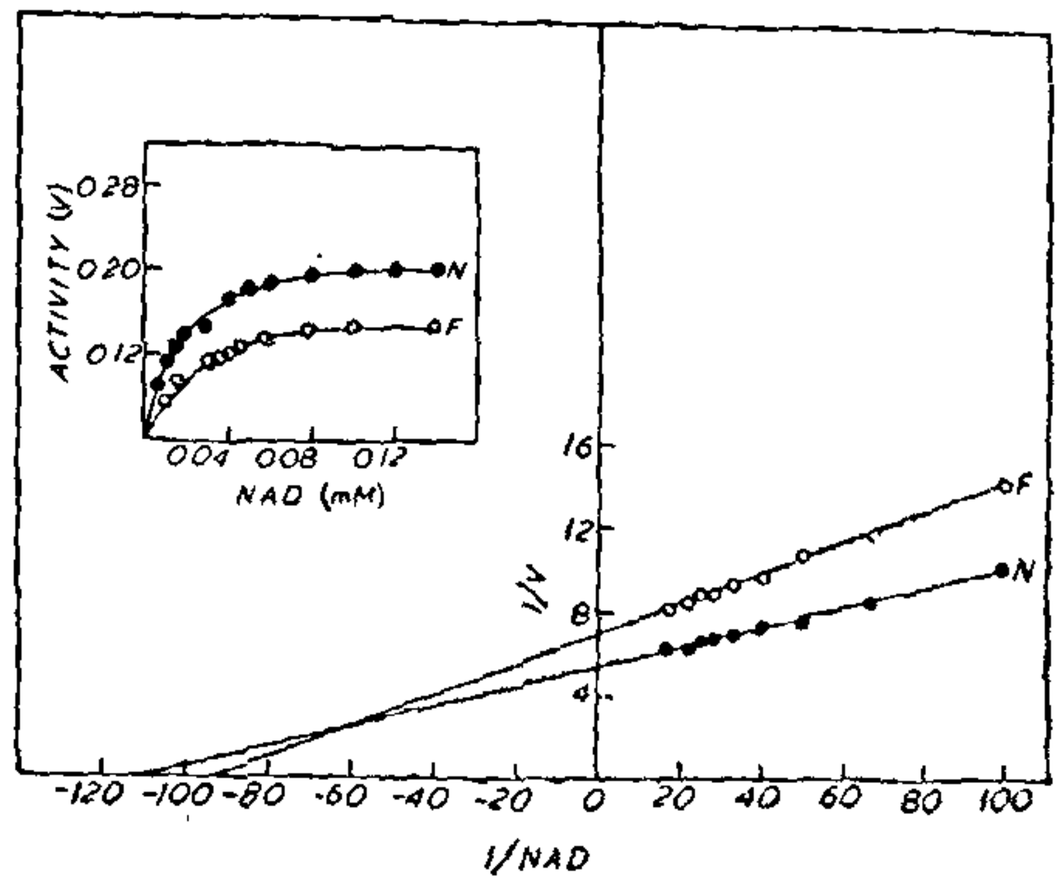

FIG. 2. Double reciprocal plots of co-factor (NAD) *versus* glutamate dehydrogenase activity of normal (N) and fatigued (F) muscles along with the co-factor *versus* activity curves.

while Km showed 22·2% increment suggesting decreased enzyme co-factor affinity. The affinity of the enzyme decreases for the glutamate (substrate) and NAD (co-factor) but the decrement was more with glutamate than with NAD in the fatigued muscle. Thus in the fatigue metabolism, the enzyme loses its affinity more for glutamate than for NAD, as evinced by increased Km values, suggesting that enzyme-substrate affinity is more affected than enzyme co-factor

affinity. Lower Kp values for co-factor in comparison with the Km values of glutamate, observed in both normal and fatigued muscles in the present investigation, indicated a preferential binding of co-enzyme which is in agreement with the earlier reports[23–25].

The catalytic efficiency of an enzyme is often measured in terms of the values of activation energy. Lower the activation energy, higher is the catalytic efficiency of the enzyme and *vice versa*. The activation energy values (ΔE) were found to be higher for the fatigued muscle enzyme (Table II) which suggests that the fatigued muscle enzyme is not catalytically as efficient as normal muscle enzyme.

TABLE II

Activation energy values for glutamate dehydrogenase in normal and fatigued muscles of frog

(Values are expressed in Cals/mole)

Sl. No.	Temperature range in °C	Activation energy	
		Normal	Fatigued
1.	25–30	20,072	25,171
2.	30–35	15,032	18,824
3.	35–40	5,829	7,788

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