IN VITRO EFFECTS OF ORGANOCHLORINE PESTICIDE (DDT) ON CATALYTIC POTENTIAL OF SDH IN GASTROCNEMIUS MUSCLE OF FROG, *RANA HEXADACTYLA*

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ABSTRACT

The effect of organochlorine pesticide on catalytic potential of succinic dehydrogenase enzyme (EC 1.3.99.1) in gastrocnemius muscle of frog, *Rana hexadactyla*, has been studied. DDT (2, 2, bis (*p*-chloro phenyl) 1, 1, 1-trichloro ethane) inhibited the enzyme activity to various degrees and also altered the Michaelis Menten constant (Km) of the enzyme. The activation energy for the enzyme was increased by the DDT Low V_{max} and high Km obtained for the enzyme suggest decreased enzyme-substrate affinity and masking of active sites by DDT.

INTRODUCTION

ORGANOCHLORINE pesticide was known to be toxic to various animal species, inhibiting the microsomal ATP-phosphohydrolases[1]. DDT and other chlorinated hydrocarbon insecticides inhibit the activity levels of Mg^{++}, Na^{+}, K^{+} dependent ATPases as well as actomyosin ATPases of nerves, muscles and other tissues[2,3,4]. DDT also causes the depletion of carbohydrate reserves, the loss of body weight and general increase in blood amino nitrogen content[5,6]. It upsets the oxidative metabolism by inhibiting succinate oxidative system and has a specific effect on the cyanide sensitive respiration by inhibiting the cytochrome oxidase[7,8].

Since muscle is known to depend on Krebs citric acid cycle for its energy requirements and SDH is a key enzyme of the same cycle, it is felt desirable to study the effect of this pesticide on kinetic parameters of the enzyme so as to assess the specific impact of this organochloride on the catalytic potential of the enzyme.

MATERIALS AND METHODS

The gastrocnemius muscles of frog, *Rana hexadactyla*, were excised from both the legs with least injury after pithing the animal and a 10% (W/V) homogenate was prepared in 0·25 M sucrose solution at 5° C. The extract was centrifuged at 2,500 rpm for 15 minutes and the supernatant was used for the assay of SDH. The activity is estimated by the method of Nachlas *et al.*[10] as modified by Pramilamma *et al.*[9] in the presence of 20 μ moles of DDT (as described by Desaiah *et al.*[11]) and the enzyme activity is expressed in μ moles of formazan/gm wet weight of tissue/hr. The maximal velocities (V_{max}), Michaelis Menten constants (Km) and inhibitor constant (Ki) were calculated by the method of least squares. The activation energy values were determined as given by Rao[12].

RESULTS AND DISCUSSION

An enzyme concentration of 50 mg, and 30 minutes of incubation time were selected for the present study after due standardization which ensures initial velocity of the enzyme catalysis. Absence of electron acceptor INT (2-*p*-Ido phenyl-3-nitrophenyl tetrazolium chloride), buffer, enzyme extract and substrate, yielded negligible activity of the enzyme indicating specificity of reacting system, for the SDH in extract at physiological pH 7·4.

The activity levels of the SDH in the gastrocnemius muscle homogenates with and without (experimental and control respectively) DDT was determined at 37° C at different substrate concentrations. Substrate concentration *versus* velocity plots for SDH of the control and the experimental mixtures revealed that the enzyme activity was linear with the substrate concentration following a first order reaction upto 40 μ moles, then onwards, the reaction entered zero order phase showing the abolition of substrate dependency indicating the saturation of the enzyme at 40 μM substrate. Lineweaver Burk plot (Fig. 1) for the SDH activity in the control and experimental mixtures showed variation in the kinetic parameters like maximal velocity (V_{max}) Michelis Menten constant (Km), intercept and slope (Table I). V_{max} values were 5·406 and 4·000 μ moles/gm wt/hr in the control and experimental tubes respectively, indicating that maximal velocity of SDH had a loss in the velocity potential suggesting significant masking of active sites in the presence of DDT as compared to control. The slope and intercept values also showed increment in the experimental when compared to the control mixtures.

The decrement of activity levels of SDH by DDT is envisaged by comparing the Km, which measures the affinity of the enzyme for substrate as well as the rate of breakdown of ES complex which forms the basis for the catalytic potential. The Km values of SDH for the substrate was 1·021 and 2·222 μM (117·63% increment) for control and experimental mixtures respectively. The alterations of both Km and V_{max} values of the enzyme by DDT indicated that this organochlorine pesticide might be reducing the ES complex formation by decreased enzyme substrate

TABLE I

Effect of DDT on SDH activity of frog gastrocnemius muscle

S.No.	Samples	Kinetic Parameters				
		Slope	Intercept	Km (μM)	V_{max}	Ki (μM)
1.	Control	0·1889	0·1850	1·021	5·406	
2.	With DDT	0·5555	0·250	2·222	4·000	18·98
	% Difference	194·1	35·135	117·63	26·01	

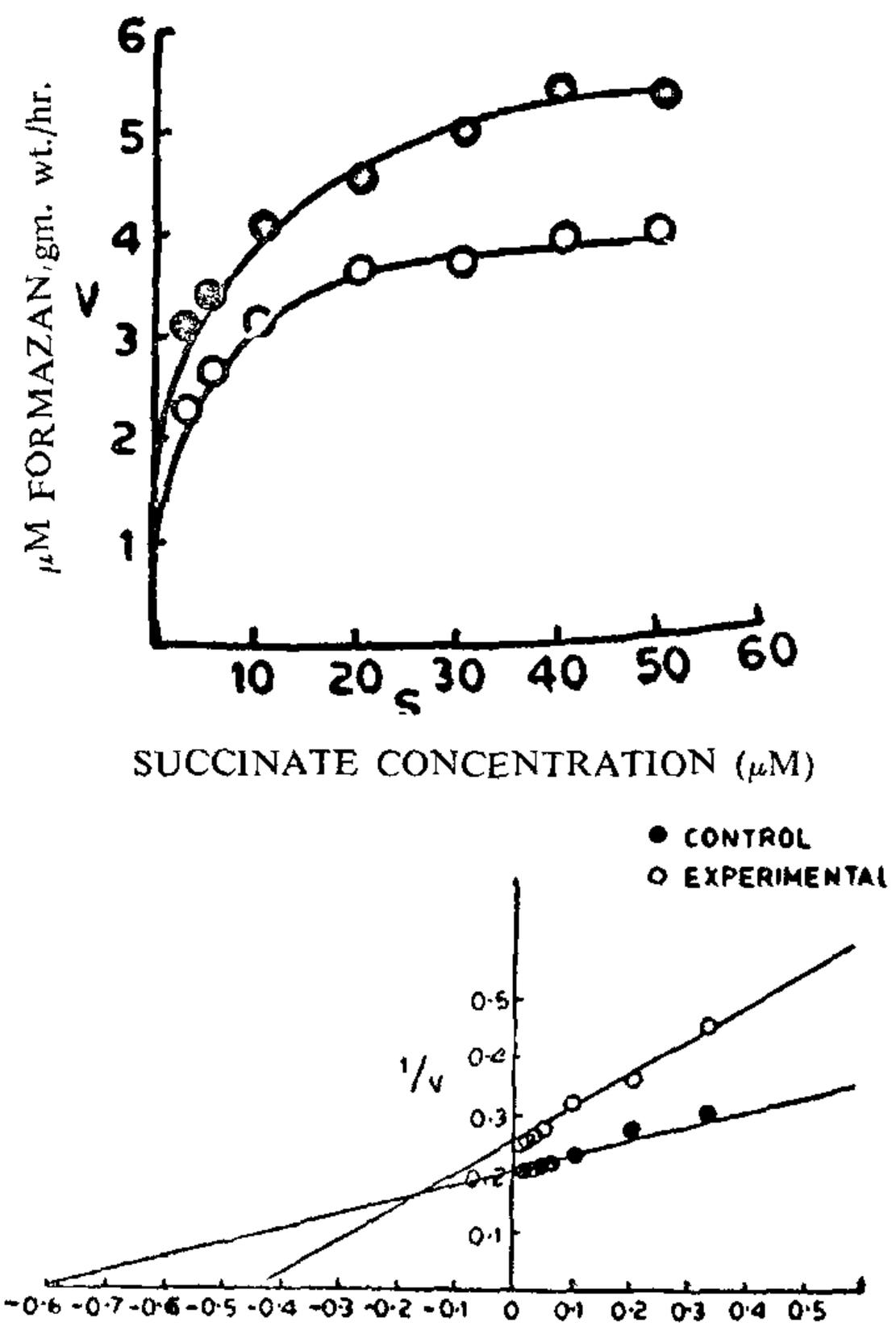

FIG. 1. Lineweaver-burk plot showing mixed type of inhibition exerted by DDT on SDH in gastrocnemius muscle of frog.

affinity and thereby indicating an alteration in the kinetic properties of the enzyme as well as total kinetic potential. Thus it appears that DDT may be imposing a sort of mixed type of inhibition on the SDH enzyme in the homogenate. The inhibition exerted by DDT might have modulated the enzyme activity in such a way as to impose restriction on the enzyme in the depletion of the physiological substrate levels at reduced rate. Though the general classification of inhibition is under mixed type, a further insight reveals a peculiar trend. From the per cent increase of Km (Table I) as compared with the decrement of V_{max}, it is presumed that the DDT inhibition tends to be more of a competitive type of inhibition, rather than the non-competitive pattern on the enzyme.

TABLE II

Effect of DDT on activation energy values of SDH in the gastrocnemius muscle of frog

S. No.	Temperature Range *in* °C	Activation energy (cals/mol)	
		Control	*Experimental*
1.	20·25	1935·3	4746·6
2.	25·30	1742·0	3595·9
3.	30·35	1685·0	1957·8
4.	35·40	1288·0	1358·5

The activation energy showed an increment in the catalysis of the enzyme in the presence of DDT (Table II). The catalytic efficiency is often measured in terms of the decrease in the activation energy values lowering the activation energy meaning higher efficiency of the enzyme and *vice versa*. The present findings show that the activation energy barrier is increased by DDT. Thus the toxic effect of DDT has a bearing at the molecular level involving enzyme kinetic parameters.

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POLAR BRILLIANT CRIMSON AS A STAINING DYE FOR ELECTROPHORETICALLY SEPARATED PROTEINS

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ABSTRACT

A new staining procedure, using polar brilliant crimson for electrophoretically separated protein fractions, is described which possesses the advantages of simplicity, sensitivity, stability of dye-protein complex and adaptability to elution and densitometric measurements.

INTRODUCTION

MANY procedures have been described for protein stains that colour protein components after electrophoresis. The stains used include bromophenol blue[1], amidoschwarz 10B[2], azocarmine B[3], lissamine green SF[4], bromocresol green[5], ponceau 2R[6], neococeine-acilan scarlet[7], nigrosine[8], ponceau S (fast ponceau 2B)[9] and procion brilliant M–RS[10]. The present report describes a new technique using the dye, polar brilliant crimson (Suhrid Geigy), for staining the protein components after electrophoresis with applicability of densitometric measurements and elution analysis.

MATERIALS AND METHODS

Paper electrophoresis.—Fractionation of serum proteins was accomplished by using barbitone-sodium barbitone buffer of pH 8·6 with 0·05 ionic strength.

Agar gel electrophoresis.—Agar shreds (50 mg) were added to 7·5 ml of barbitone-sodium barbitone buffer and heated. The molten agar (1 ml) was layered on 2·5 × 7·5 cm microscope slide (or polyester film) and allowed to set for 40 to 60 min. After application of serum, a constant current at 200 volts was applied for 30 min and at the end of the run the slide (or polyester film) was placed in methanol for the fixation of the protein bands. The slide (or film) was finally dried at room temperature.

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Dye solution.—0·5 gm of polar brilliant crimson (PBC) in 20 ml ethanol was made up to 100 ml with 3% sulphosalicylic acid.

Clearing solution—95% methanol containing 10% acetic acid V/V.

Eluting solution.—1% sodium carbonate in 50% aqueous ethanol.

The protein components separated on the paper and on the agar gel were stained with the dye for 10 minutes at room temperature, washed for 4 min in two changes of clearing solution, then rinsed with methanol and allowed to dry at room temperature. The quantitative evaluation of the stained protein components was made by scanning in Photovolt Densitometer Model 525 and by photometric determination in Spectronic 20, of the dye eluted from the segregated fractions, at 535 nm. The electrophoretically separated protein fractions were cut out and eluted with 6 ml of eluting solution for 30 minutes, mixing at intervals

RESULTS AND DISCUSSION

By the use of the dye, polar brilliant crimson, the protein components in electropherogram show as red bands against clear background without any free dye adsorbed on the media. The dye is sensitive to stain all protein fractions and the patterns do not show any deterioration even after 7 years indicating that the dye-protein complex is quite stable. The