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Study of *Salmonella* endotoxin on the changes in lipid-protein interactions of membranes using Arrhenius plots of cetylcholinesterase as a tool

M. K. RAY, A. MITRA, B. MUKHERJEE and G. C. CHATTERJEE*
Department of Biochemistry, University College of Science, 35, Ballygunge Circular Road, Calcutta 700 019, India

Abstract. Lipopolysaccharides of *Salmonella typhimurium* inhibit the activity of acetylcholinesterase *in vitro* in both synaptosomal and erythrocyte membranes. Arrhenius plots show that the transition temperatures of membrane bound acetylcholinesterase are significantly reduced in the presence of lipopolysaccharides, and the activation energies above and below transition temperature have increased with the lowering of transition temperature. These results indicate that an alteration in the fluidity of the phospholipid layer of the membranes, may be responsible for the membrane-specific effect of lipopolysaccharides on acetylcholinesterase activity.

Keywords. Membrane; acetylcholinesterase endotoxin; transition temperature; inhibition.

Introduction

Gram negative bacterial toxins are broadly represented by lipopolysaccharides (LPS) which elicit their toxic affects after attachment to cell-associated extracellular receptors like the lipoglycoprotein on erythrocyte membrane (Springer and Adye, 1977). LPS administration is known to change the fluidity of hepatic plasma membrane (Conde *et al.*, 1981) and there is a close interrelationship between membrane enzyme activity and membrane fluidity (Liu *et al.*, 1983). LPS play a significant role in the development of pathophysiology of endotoxic shock at the cellular level, by decreasing membrane fluioty (Onji and Liu, 1981). Solomonson *et al.* (1976) demonstrated that alteration of plasma membrane fattyacyl composition *in vivo* results in altered properties of $(Na^+ + K^+)$-ATPase associated with this membrane. They suggested that the fattyacyl groups as well as the polar head groups of phospholipids are important determinants of enzyme activities. Membranes undergo gel to liquid crystal-phase transition which are dependant on the fatty acid composition of the membrane. Kimelberg and Mayhew (1975) showed that the activity of certain membrane bound enzymes was increased in virally transformed cells and when this occurred, the transition temperature (TT) and the activation energy of the enzymes were altered. The apparent activation energy of most soluble enzymes is constant between 0°C and 37°C. However, mammalian membrane bound enzyme systems show a large change in activation energy over this tempe -rature interval. Kimelberg and Papahadjopoulos (1972) suggested that changes in the activation energy of membrane bound ATPase was due to a lipid phase transition. It has been established that in most cases membrane bound enzymes show a TT in their Arrhenius plots. It has further been proved that the existence of a TT is due

*To whom all correspondence should be addressed.

Abbreviations used: LPS, Lipopolysaccharides; TT, transition temperature; AchE, acetylcholinesterase; DTNB, 5,5-dithio bis (2-nitrobenzoic acid).

not to any effect of temperature on the protein moiety of the enzyme, but to changes in the physical properties of the membrane lipids surrounding and interacting with the enzyme (Grisham and Barnett, 1973). Acetylcholinesterase (AchE), one of the most important brain enzymes, is believed to be involved in impulse transmission at cholinergic synapses (Rieger and Vigne, 1976). The activity of this peripheral-extrinsic enzyme has been found to be influenced significantly by membrane phospholipids (Beauregard and Roufogalis, 1977; Sihotang, 1976). In this laboratory, studies on different aspects of LPS isolated from *Agrobacterium tumefaciens* and *Salmonella typhimurium* have been carried out (Banerjee *et al.,* 1981, 1983; Haldar *et al.,* 1984; Mitra *et al.,* 1985; Ray *et al.,* 1985). In the present study however the possible changes in lipid-protein interactions of the enzyme AchE of isolated brain synaptosomal and erythrocyte membranes during the treatment with *S. typhimurium* LPS have been determined.

Materials and methods

The chemicals used in this study were of the analytical grade, commercially available. The LPS of *S. typhimurium* was purchased from Sigma Chemical Company, St. Louis, Missouri, USA.

Experimental animals

Male swiss albino mice weighing 20 ± 22 g maintained on laboratory diet (Chatterjee *et al.,* 1973) and water *ad libitum* were used in the experiments.

Membrane preparation

Mice brain synaptosomal membrane was prepared according to the differential method of Gray and Whittaker (1982) as modified by Bradford *et al.* (1973). Erythrocyte ghost was prepared as described by Kunimoto and Miura (1985).

Enzyme assay

AchE activity was determined spectrophotometrically according to the procedure of Ellman *et al.* (1961). The final assay medium totalling 3 ml consisted of 0·29 mM 5,5-dithio bis (2-nitrobenzoic acid) (DTNB), 0·5 mM acetylthiocholine iodide and 0·05 ml of the enzyme preparation in a medium of phosphate buffer. To study the *in vitro* effect of LPS, the membrane preparation was preincubated with 50 μg/ml of LPS at 37°C for 20 min, prior to addition of substrate. The rate of change of colour was measured at 412 nm. Assays were performed at temperatures varying from 10°–40°C at 2–5 degree intervals.

Protein assays

The protein content of the enzyme preparations was estimated according to Lowry *et al.* (1951) using bovine serum albumin as standard.

Arrhenius plots

To construct the Arrhenius plots, square root analysis of the data was first carried out and then the logarithms of the corrected specific activity values at each temperature were plotted against the reciprocal of absolute temperature. The value of transition temperature was read directly from the plot.

Estimation of activation energy

The Arrhenius equation was utilised to estimate the activation energies of the enzyme above and below the transition temperatures.

Statistical significance

The Statistical significance of differences between mean values of experimental and control was determined by Student's *'t'* test.

Results

The activity of brain synaptosomal membrane bound AchE was measured at various temperatures (10°C–40°C) and it is found that the activity of the enzyme was suppressed with rise in temperature, in the presence of *S. typhimurium* LPS as compared against the control (figure 1). Figure 3 depicts the activity of erythrocyte membrane bound AchE under similar experimental conditions where inhibition in the enzyme activity with increase in temperature in the presence of LPS has also

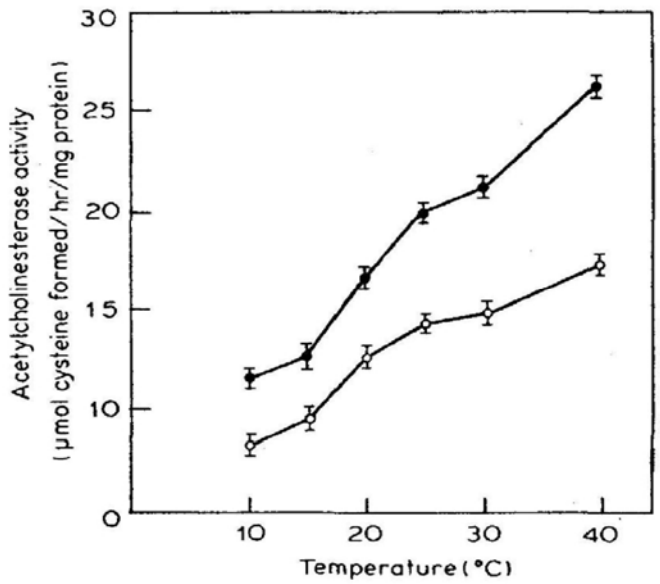

Figure 1. Effects of temperature on AchE activity in the brain synaptosomal membrane in the absence (●) and presence of 50μg/ml (O), LPS

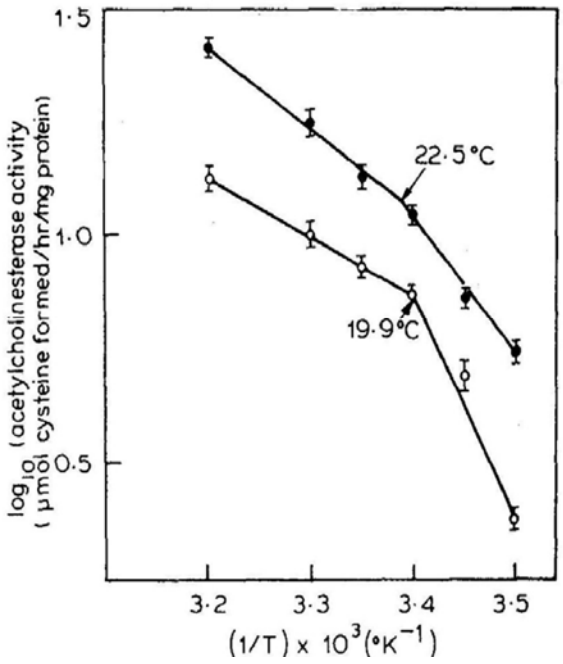

Figure 2. Arrhenius plots of AchE of brain synaptosome in the absence (●) and presence of 50 μg/ml (O), LPS

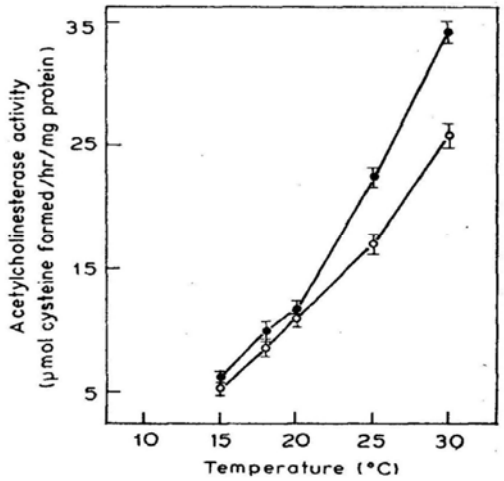

Figure 3. Effect of temperature on the erythrocyte membrane bound AchE in the absence (●) and presence of 50 μg/ml (O), LPS.

been observed although the inhibition in the erythrocyte membrane enzyme is lower when compared with that of the synaptosomal membrane enzyme. However, the activity of the enzyme rises with temperature *i.e.* lowest at 10° and highest at 40°C.

When the results are presented in the form of Arrhenius plots discontinuities in the

slope became apparent irrespective of the absence or presence of LPS. Bigures 2 and 4 are the corresponding Arrhenius plots of AchE activity of synaptosomal and erythrocyte membranes respectively. Examination of the plots show that the normal TT of synaptosomal AchE is 22·5°C which is lowered to 19·9°C on being treated with 50 μg/ml *S. typhimurium* LPS *in vitro.* The same amount of LPS lowered the TT of erythrocyte AchE to 18·7°C from 21·1°C.

Table 1 shows the apparent activation energy above and below the transition temperature of membrane bound AchE activity in erythrocytes and synaptosomal fraction.

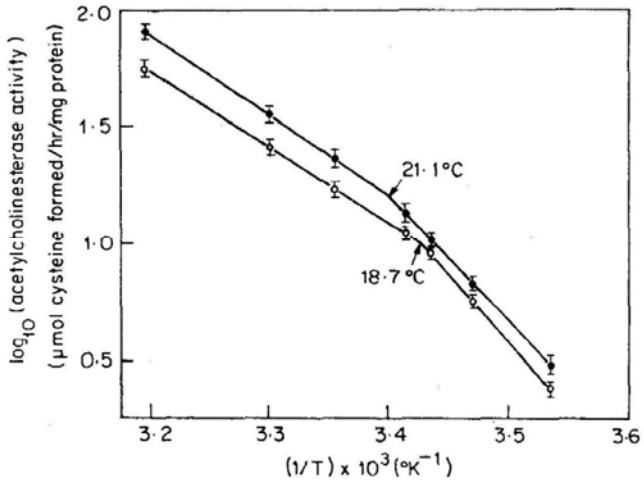

Figure 4. Arrhenius plots of erythrocyte membrane bound AchE in the absence (●) and presence of 50 μg/ml (O), LPS.

Each point represents in the mean ± S.D. of 5 experiments each on a different animal.

Table 1. *In vitro* effect of *S. typhimurium* LPS on transition temperature and apparent activation energy* of synaptosomal and erythrocyte membrane bound AchE activity.

Membrane AchE	LPS concentration (μg/ml)	Transition temperature	Activation energy (Kcal/mol)	
			Below TT	Above TT
Synaptosome	0·0	22·5	12·937 ± 0·77	2·5 ± 0·15
	50·0	19·9	11·9 ± 0·831	4·0 ± 0·28[a]
Erythrocyte	0·0	21·1	25·25 ± 1·515	15·633 ± 0·93
	50·0	18·7	28·264 ± 1·695[b]	15·059 ± 0·9

*Activation energy was calculated from the slope of the lines of figures 2 and 4.
Each result represents mean value ± Standard deviation of 5 separate determinations.
Mean value significantly different from the control group [a]P<0·001, [b]P<0·05.

Discussion

AchE, like many other membrane-bound enzymes undergoesa dramatic change in apparent activation energy between 10°–40°C. Although a number of explanations

have been offered for this phenomenon, lipid protein interactions often have been suggested as playing a major role. The present data suggest that lipid phase changes can be correlated to Arrhenius plot breaks. The work of Kimelberg (1975) and Warren *et al.* (1975) on reconstituted membrane systems containing ($Na^+ + K^+$) - ATPase and Ca^{2+}-ATPase lends support to the above assumption. AchE is a peripheral extrinsic phospholipoprotein and the role of lipid especially phospholipids is vital for this enzyme activity (Mitchell and Hanahan, 1966). The lipid bilayer is believed to be the site of action of LPS and other lipophilic compounds on biomembranes (Tanaka, 1974). Hence it is reasonable to assume that variation of lipid composition of different membranes (Solomonson *et al.,* 1976) as well as their structural organisation (Nemat-Gorgani and Meisami, 1979) may be responsible for the membrane-specific efect of LPS on AchE activity. There is indirect evidence that membrane alteration may involve a lipid change and this idea has been expressed recently by Londesberg (1980) who proposed that the higher apparent activation energy at low temperatures may result from the addition of an enthalpy component associated with a lipid phase transition around the enzyme. The sharp discontinuity in the Arrhenius plot is found at the TT which is the crystalline to liquid-crystalline phase transition temperature of membrane lipids. The TT of synaptosomal AchE is found to be 22·5°C and that of erythrocyte AchE is 21·1°C. LPS-induced lowering of TT in both membranes indicates that membrane lipid phase transition takes place at a lower temperature in the presence of LPS and this probably is brought about by an alteration in the fluidity of the phospholipid mono- and bilayer environment (Papahadjopoulos and Kimelberg 1974, Heron *et al.,* 1980).

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