then again, it may be that the intranuclear calcium takes part in intracellular calcium kinetics. Whether the changes are related to mechanical muscle activity or are an expression of adaptation processes remains unclear.

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Résumé. L'analyse par microsonde électronique des cellules musculaires montre de hautes concentrations de calcium dans les noyaux; la concentration peut être modifiée pharmacologiquement ou par «pacemaker» électrique

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Organophosphate-Detoxicating Enzymes in *E. coli*. Gelfiltration and Isoelectric Focusing of DFPase, Paraoxonase and Unspecific Phosphohydrolases

Toxic organophosphates, commonly known as insecticides or as nerve gases, are inhibitors of cholinesterases, thereby blocking nerve transmission[1]. Some hydrolytic enzymes ('phosphorylphosphatases') are capable of splitting the acidanhydride bond of organophosphate poisons, forming non-toxic organophosphorus acid[2,3]. The following paper describes the enzymatic organophosphate detoxication by *E. coli* phosphohydrolases. Two detoxicating enzymes were found, a DFPase (E.C. 3.8.2.1) and a paraoxonase (arylesterase, E.C. 3.1.1.2).

DFPase-activity was determined by 3 methods with di-isopropylfluorophosphate (DFP) as substrate: titrimetrically[4], manometrically[5] or by measuring the liberated fluoride with an ionsensitive electrode[6]. Paraoxonase was tested colorimetrically with 0,0-diethyl-0-*p*-nitrophenylphosphate (paraoxon)[7]. Alkaline and acid phosphatase were measured colorimetrically with *p*-nitrophenylphosphate in 0.2 *M Tris*-buffer at pH 9 and pH 6, the absorbance at 405 nm was read after diluting samples with 1 *M* sodium hydroxide. Phosphodiesterase determinations were performed with *bis-p*-nitrophenylphosphate at pH 8 in the same way. All tests were performed at 25 °C, activity is expressed as 1 μmole substrate hydrolyzed per min. *E. coli* K_{12}sr extracts were prepared according to MCILWAIN[8]. The extract was fractionated by gelfiltration on a Sephadex G-200 column, the elution diagram for protein und DFPase is shown in Figure 1. The distribution coefficients (K_d-valus) of the 2 DFPase peaks were calculated[9] and compared with those of other phosphohydrolases in a second experiment on the same column (Table I).

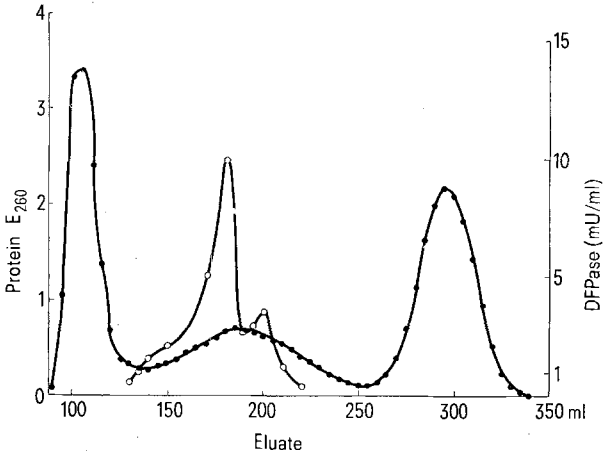

Fig. 1. Gelfiltration of *E. coli* extract on Sephadex G-200 (2.5 × 73 cm). 4 ml extract (2 g wet cells) containing 73 mg protein and 562 mU DFPase were fractionated at 1°C, flow rate: 0.2 ml/min. ●, protein ($E^{1\,cm}_{260}$); ○, DFPase (mU/ml).

DFPase and paraoxonase could clearly be seperated by gelfiltration; no overlapping activity was detected: DFPase fractions did not hydrolyze paraoxon and vice versa. There was no hydrolysis of *p*-nitrophenylphosphate at acid or alkaline pH or of *bis-p*-nitro-phenylphosphate by either the DFPase or paraoxonase containing fractions. The phosphohydrolases in *E. coli* extracts were additionally subjected to free preparative isoelectric focusing[10,11]. Four DFPase peaks with isoelectric points of 5.3, 5.7, 6.1 and 7.8 were found (Figure 2). The isoelectric points of the other phosphohydrolases were determined in an identical way in a different experiment, the isoelectric points are listed in Table II. The phosphohydrolases of *E. coli* showed similar or even identical isoelectric points, so, in contrast to the gelfiltration experiment, they could not be separated by this method.

Coli DFPase has a low substrate affinity (Km 1.7×10^{-2} *M*/l for DFP) and shows pure MICHAELIS-MENTEN kinetic (Figure 3). The pH-dependence of *coli* DFPase is shown

Table I. Distribution coefficients (K_d-valus) of *E. coli* phosphohydrolases on Sephadex G-200 (2.5 × 73 cm)

Enzyme	K_d-valus		
DFPase	0.21	0.31	
Paraoxonase	0.11	0.55	0.66
Acid phosphatase	0.39		
Alkaline phosphatase	0.42		
Phosphodiesterase	0.40		

Table II. Isoelectric points of *E. coli* phosphohydrolases determined by free preparative isoelectric focusing in 2% ampholine, pH 3–6

Enzyme	Isoelectric points			
DFPase	5.3	5.7	6.1	7.8
Paraoxonase	5.3	5.6	6.2	
Acid phosphatase	4.9	5.3	6.4	
Alkaline phosphatase	4.8	5.3		
Phosphodiesterase	5.0	5.3	5.6	6.3

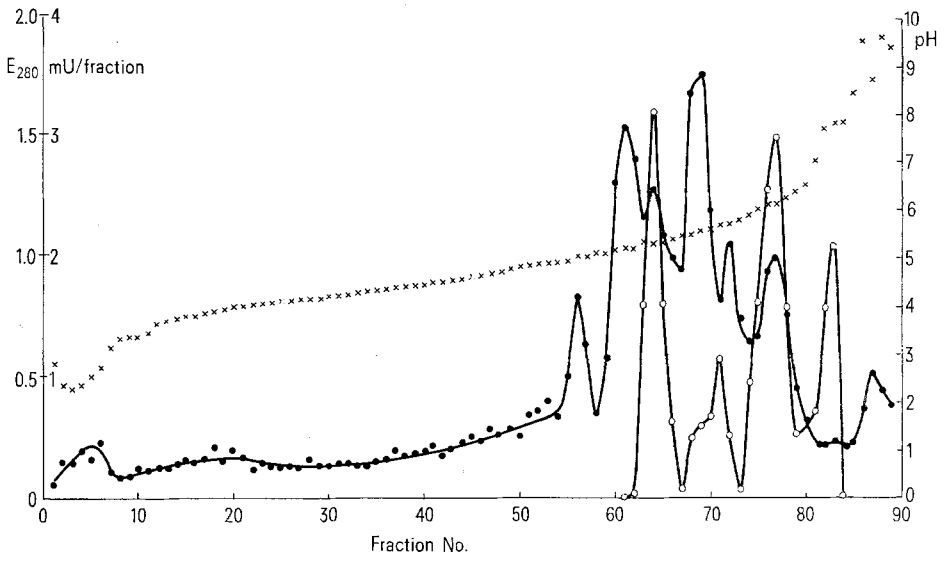

Fig. 2. Preparative free isoelectric focusing of *E. coli* extract (1.8 ml extract, containing 324 mU DFPase and 29 mg protein + 12 ml 2% ampholine, pH 3–6, 41 h, 1000 V. ×, pH; ●, protein ($E\,\frac{1\,cm}{260}$); ○, DFPase (mU/fraction).

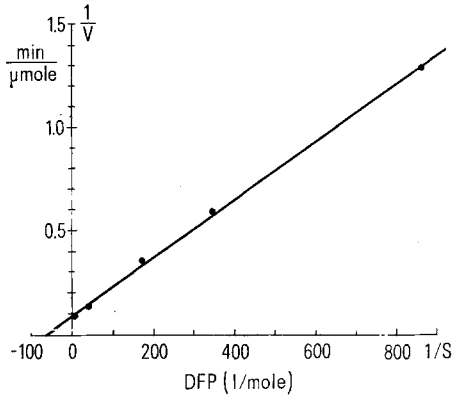

Fig. 3. LINEWEAVER-BURK-plot of DFP-hydrolysis by *E. coli* DFPase. Automatic titrimetric assay in 10 ml 0.1 *M* NaCl with 200 µl extract (10 mg protein), pH 8, 25 °C.

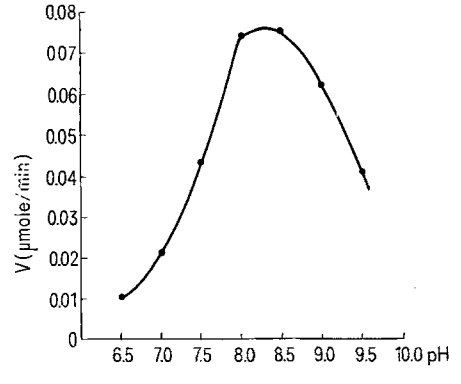

Fig. 4. pH-dependence of *E. coli* DFPase. Automatic titrimetric method at 0.1 *M* substrate concentration in 0.1 *M* NaCl with 200 µl extract containing 10 mg protein.

in Figure 4, the pH optimum is at 8.3. The corresponding kinetic constants for paraoxonase in *E. coli* tested with paraoxon are Km 5×10^{-3} *M/l*, the pH optimum is at 9.3. The extracted phosphorylphosphatases are rather unstable, losing about 10 to 30% activity per day at room temperature.

The instability of the enzymes and the low substrate affinity make *E. coli* DFPase and paraoxonase unsuitable tools for preparing efficient enzymatic protectors against organophosphate poisoning of mammals.

Zusammenfassung. Die Entgiftung von Organophosphaten durch *E. coli*-Phosphophydrolasen wurde untersucht und eine DFPase (E.C. 3.8.2.1) und eine Paraoxonase (E.C. 3.1.1.2) nachgewiesen. Die Trennung der Phosphohydrolasen erfolgte durch Gelfiltration und die isoelektrischen Punkte wurden in freier, präparativer Fokussierung bestimmt. Ausserdem wurden Substrat- und pH-Abhängigkeit untersucht.

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