Improved Method of Large Scale Purification of Acetylcholinesterase from the Electric Eel (*Electrophorus electricus*) by Affinity Chromatography[1]

Although long sought after, the large scale purification of acetylcholinesterase (AChE, E.C. 3.1.1.7)[2] from *Electrophorus electricus*, a rich source, was not reported until 1967[3] by conventional chromatography. More recently, BERMAN and YOUNG[4], and others[5,6] succeeded in purifying eel AChE using affinity chromatography with varying degrees of effectiveness. We have reported the purification of AChE from mammalian brain using affinity chromatography[7–9]. We now report the large scale complete purification of the eel enzyme by this procedure. The present method provides a simpler, more convenient, more efficient scheme for producing large amounts of the enzyme.

Materials and methods. AChE activity was measured at 23 °C by the titrimetric method with 0.01 *N* NaOH in media[4] containing 4 m*M* ACh, 100 m*M* NaCl and 40 m*M* $MgCl_2$ at pH 8. Protein was determined by the LOWRY[10] method.

The side arm of affinose-202 was extended to twice its length according to the method of CUATRECASAS[11]. *m*-Trimethylammoniumaniline was prepared as reported previously[9] while the *para* analogue was prepared according to the method of BERMAN and YOUNG[4].

Dialyzed, crude eel AChE (Type III, 70 units/mg protein, Sigma Chemical Co.) was used essentially as described earlier[9]. The ligand *m*-Trimethylammoniumaniline was used since the binding and subsequent recovery of the AChE was higher than with the *para* analogue. Following enzyme binding the column was washed with 100 ml of 30 m*M* *Tris*-HCl buffer, pH 8.0, followed by 460 ml of 100 m*M* NaCl containing 40 m*M* $MgCl_2$, *Tris* buffered to pH 8.0.

Gel filtration and molecular weight determination was carried out on a standardized Sephadex G-200 column (1.3 × 100 cm) as described previously[12] using the method of WHITTAKER[13].

Samples containing 200 µg of crude and 30 µg of purified enzyme were subjected to electrophoresis at 1 mA/gel for 2.5 h on 6% polyacrylamide gels (PAGE) as described by HEDRICK and SMITH[14]. Gels were stained with 0.5% Coomassie Blue in 12.5% TCA as described by CHROMBACH et al[15]. Protein bands were stained for AChE activity by an adaptation of the method of URIEL[16].

Results and discussion. The Table shows that 99% or more of the crude AChE was bound to the 5–10 ml bed volume of affinity gel after exhaustive washing. The recovery of AChE eluted with edrophonium chloride varied from 72% to 90% with increasing amount of enzyme applied. The recovered AChE always had a specific activity of > 950 m*M* ACh hydrolyzed/mg protein/h.

The pattern of AChE elution with edrophonium chloride shown (Figure 1) is for 2,400 units of AChE applied to 7 ml of affinity gel. Fraction 0 represents the last fraction of the 560 ml wash. Active fractions were combined and concentrated to a volume of 1 ml before application to a Sephadex G-200 column. A single peak of enzyme activity was observed in conjunction with a single protein peak. 70% of the enzyme was recovered in this step. The M. W. of the AChE peak was found to be 265,000.

Crude eel AChE (200 µg) on PAGE showed 4–5 bands of protein with only 1 exhibiting AChE activity. PAGE of the AChE preparation (30 µg) after affinity chromato-

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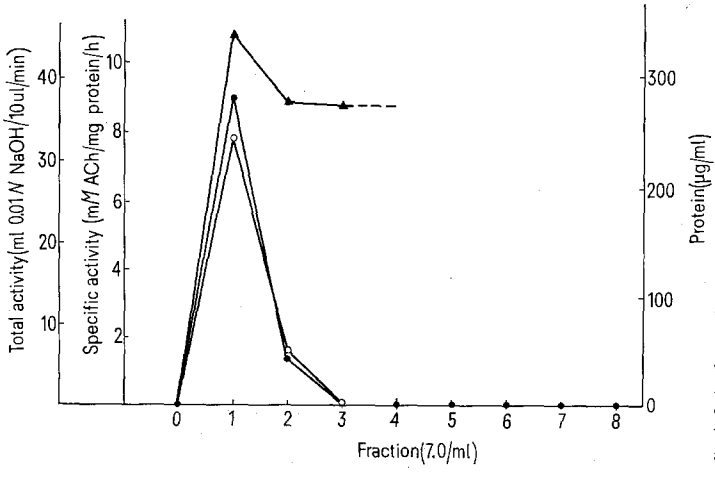

Fig. 1. Affinity chromatographic elution pattern. Fractions 1–8 were eluted with edrophonium chloride then dialyzed exhaustively. Symbols: ○—○, protein (µg/ml); ●—●, total AChE activity; ▲—▲, specific AChE activity (m*M* ACh hydrolyzed/mg protein/h × 10^{-2}). After fraction 2, protein content is too low to permit accurate determination of specific activity as indicated by the extended dashed line.

Purification and recovery of AChE after affinity chromatography

AChE (units applied)	Protein (mg)	Specific activity	AChE (% bound)	AChE units recovered (%)	Protein recovered (mg)	Recovered specific activity
0,200 (4)	2.85	70	99.0	0,144 (72)	0.152	0,950
0,400 (2)	5.72	70	99.0	0,300 (75)	0.309	0,970
2,400 (1)	34.32	70	99.4	2,160 (90)	2.16	1,000
4,800 (1)	68.64	70	99.2	4,320 (90)	4.42	0,980

One unit is 1 m*M* ACh hydrolyzed/h. Numbers in parentheses under AChE units applied represent the No. of experiments. Specific AChE activity is expressed in m*M* ACh hydrolyzed/mg protein/h. Specific activity applied in each case was 70 m*M* ACh hydrolyzed/mg protein/h.

graphy shows a single protein band exhibiting AChE activity (Figure 2). Since the limit of detection of a protein in PAGE is 1 µg or less[15], the AChE obtained after affinity chromatography is more than 97% pure.

The effectiveness of the affinity gel is evidenced by the fact that the enzyme could not be eluted from the gel with 500 ml of 600 m*M* NaCl at pH 8.0 but complete elution (99%) was effected with 3–4 bed volumes of 10 m*M* edrophonium chloride in 100 m*M* NaCl.

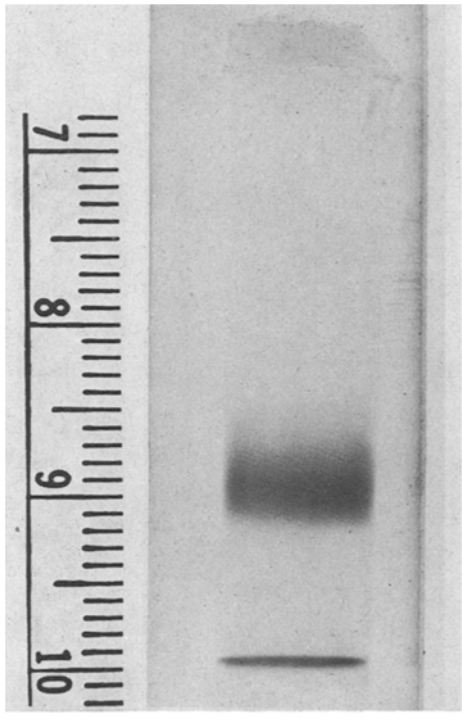

Fig. 2. Page of purified EE1 AChE. Sample contained 30 µg of protein. Lower band shows the tracking dye, Bromthymol blue.

By using a 10 ml bed volume of affinity gel, we have obtained 4.5 mg of purified AChE in a single run. In agreement with previous studies[17], the purified eel enzyme appears not to aggregate or dissociate as freely as purified brain AChE[9]. The M.W. of the enzyme as determined by gel filtration was 265,000, a value similar to that previously reported[18]. Two reports have since appeared[5,6] in which affinity chromatography was used in a similar manner for AChE purification. Enzyme recovery averaged 63 % in one case[5] and 40% in the other[6]. In the present study, recovery ranged between 70 and 90% which may be due to our method of coupling the ligand to the agarose and/or the use of edrophonium as a more specific elutant[19].

Résumé. Une méthode basée sur d'affinité chromatographique nous a permis de purifier complètement l'acétylcholinestérase des organes électriques du gymnote (*Electrophorus electricus*). L'activité spécifique de l'acétylcholinestérase ainsi établie en milligrammes dépasse 950 m*M* de substrat hydrolysé (acétylcholine)/mg protéine/h et sa pureté a été vérifiée par électrophorèse sur gel de polyacrylamide.

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A Technique for Improving Salivary Chromosome Preparations[1]

Since the initial work of Painter[2] with the polytene chromosomes of *Drosophila*, the salivary-gland chromosome smear technique has been improved by many workers[3–5]. A new modification of methods currently in use for the chromosomes makes it possible to prepare slides having better resolution and clear background. This method has been successfully employed in the studies of many genera in the family Drosophilidae[6].

The major modifications of the ususal procedures are: 1. Overstaining of the chromosomes (30 ~ 40 min in lacto-acetic-orcein), and 2. Washing the chromosomes with lacto-acetic acid at least 3 times before squashing to remove excess stain and other dirt particles.

When the preparations are analyzed by phase contrast microscopy, the main advantages of this new method are especially evident. The overstaining (longer than usual) causes the bands to darken and the thinner (faint) bands to be stained, thus improving the fine detail of the bands (Figure 1). The 'washing technique' removes excess stain especially between light bands as well as in the neighbor-