MOLECULAR FORMS OF CHICKEN ACETYLCHOLINESTERASE: EFFECT OF DENERVATION

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1. Introduction

Acetylcholinesterase (AChE: EC 3.1.1.7.) is an ubiquitous enzyme, found always in a series of forms differing for their sedimentation coefficients. In electroplax from electric fish, such as *Torpedo* and *Electrophorus*, where the polymorphism of AChE was first observed, several forms have been described [1,2]. Three forms, with respect to their sedimentation coefficient, were then found in mammalian organs [3,4]. The number and proportions of the various forms vary in different organs of the same species and from one species to another. None of these forms seems to be specific to a particular tissue or function with the exception of the highest molecular weight form, which seems to be associated with motor end plates regions [3].

In this paper we report a study of the molecular forms of AChE in different organs of the chicken. The investigation, carried out by sedimentation analysis in a sucrose gradient, includes the characterization of the number, sedimentation coefficients and abundance of the different molecular forms and the effect of muscle denervation on these parameters.

2. Materials and methods

2.1. *Chemicals*

Acetylcholine iodide was obtained from Koch Light Laboratories; 5,5'-Dithio(bis)dinitrobenzoic acid (DTNB) from Aldrich chemicals, horse alcohol dehydrogenase (4.8 s_{20w}) and β-galactosidase (16 s_{20w}) from Worthington; Triton X-100 from Prolabo; ethopropazine hydrochloride (Parsidol) from Rhone-Poulenc.

2.2. *Preparation of tissue extracts*

White leghorn chickens, 4 weeks old, were raised in the laboratory. The tissue of the various organs (brain without cerebellum, nerves, ciliary ganglia, muscles) were homogenized in a glass-Teflon potter homogenizer with extraction buffer (1 M NaCl, 0.01 M Tris–HCl pH 7, 0.05 M $MgCl_2$, 1% Triton X-100). The ratio weight to volume was generally 1 : 4; exceptionally 1 : 400 for ciliary ganglia. The homogenate was then centrifuged at 20 000 × *g* for 20 min at 4°C in a Sorvall centrifuge. The pellet was discarded, the supernatant was retained and considered as the tissue extract.

2.3. *Cholinesterase and protein assays*

Cholinesterase activity was assayed by the method of Ellman [5] at 20°C, with acetylthiocholine as substrate and 10^{-4} M ethopropazine as pseudocholinesterase inhibitor.

Cholinesterase activity will be expressed in operationally defined units: one unit equal to 1 O.D./min/1 ml Ellman reagent, 1 cm pathlength at 412 nm. One unit corresponds to the hydrolysis of 73.5 nmol of acetylthiocholine per min [10].

Protein content was estimated by the method of Lowry [6].

2.4. *Sucrose-gradient-sedimentation analysis*

The measurements of the sedimentation coefficients

of the different forms of AChE were done in a 5–20% sucrose gradient in extraction buffer (see 2.2) according to the technique of Martin and Ames [7].

2.5. *Muscle denervation*

Slow muscles (Anterior Latissimus Dorsi: ALD) and fast muscles (Posterior Latissimus Dorsi: PLD) were denervated by ligature and section of the common nerve trunk [8,9]. The denervated muscles were dissected and analysed 3 weeks after denervation.

3. Results

3.1. *Brain*

The specific activity of a brain extract is 38 units of AChE per mg of protein (280 nmol AcThCh/min/mg prot.). The solubilization yield is 235 units per gram of tissue (wet weight), which accounts for 90% of total tissue activity.

Figure 1 shows the sedimentation pattern of chick brain AChE. Two forms, with sedimentation coefficients of 11 S and 6.5 S are visible. The relative proportion of the two forms is respectively 75 and 25%.

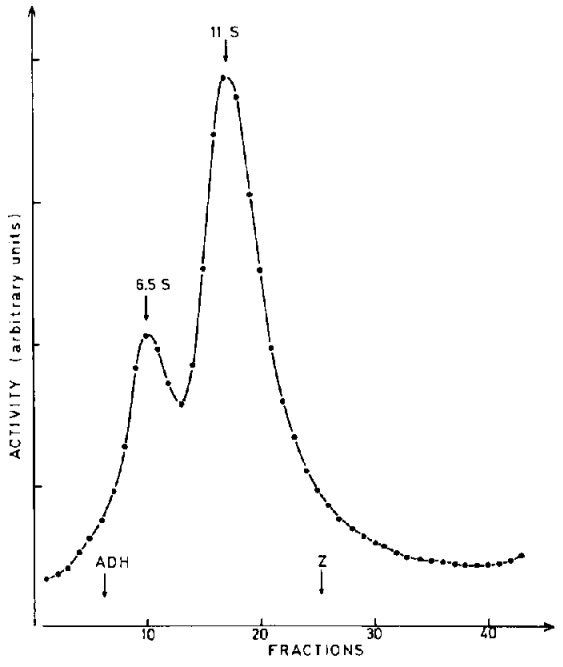

Fig.1. Sedimentation profile of AChE activity from chicken brain extract. Z, β-galactosidase; ADH, alcohol dehydrogenase.

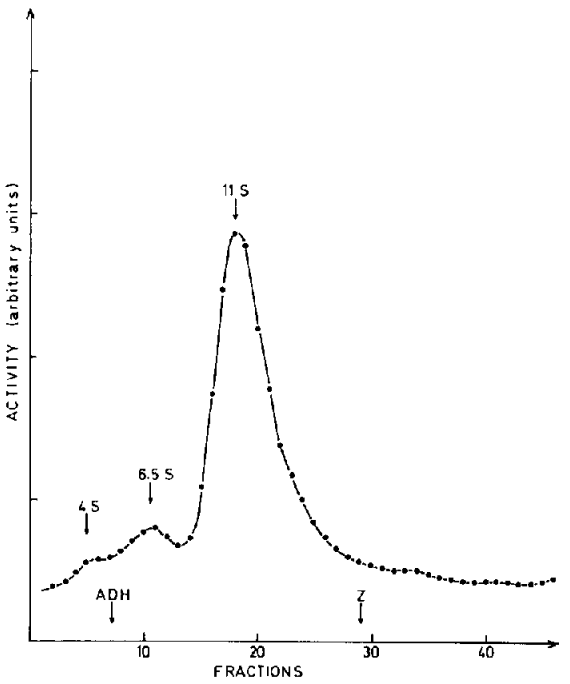

Fig.2. Sedimentation profile of AChE activity from peripheral nerve extracts; ciliary ganglion nerve and sciatic nerve. Z, β-galactosidase; ADH, alcohol dehydrogenase.

If the detergent (Triton X-100) is omitted from the extraction medium, the specific activity of the resulting brain extract, the sedimentation coefficients and the relative proportions of the two forms are unaffected, but the solubilization yield is reduced to 20% of the total tissue activity.

3.2. *Peripheral nerves*

The sedimentation profile of AChE extracted from preganglionic nerves (ciliary ganglion) is reported in fig.2. In addition to the two forms already described for brain extract (the 11 S and the 6.5 S, which account respectively for 85 and 10% of the nerve extract activity), a new form of very low molecular weight is observed (sedimentation coefficient of 4 S, 5% of the nerve extract activity). Sciatic nerve extracts yield an identical pattern.

3.3. *Muscle*

Table 1 shows the specific activity of fast (PLD) and slow (ALD) muscles (normal and denervated).

Table 1
Effect of denervation on the specific activity of muscle AChE

Muscle type	Specific activity (Units/mg of prot.)	
	Normal	denervated
Fast (PLD)	0.15	0.08
slow (ALD)	1.00	0.50

The specific activity, which is four or five times higher in the PLD than in the ALD, drops by 50% after denervation in either type of muscle.

Figure 3 shows the sedimentation profiles of AChE extracted from fast muscle, normal and denervated. The profiles of fast and slow muscle are very similar. In addition to the two forms found in the brain a new form, of sedimentation coefficient of 19.5 S, is observed in normal muscle. This high molecular weight form disappears after denervation, in both fast and slow muscle. The relative proportion of the 3 forms (19.5 S; 11 S; 6.5 S) is respectively 10, 15 and 75%.

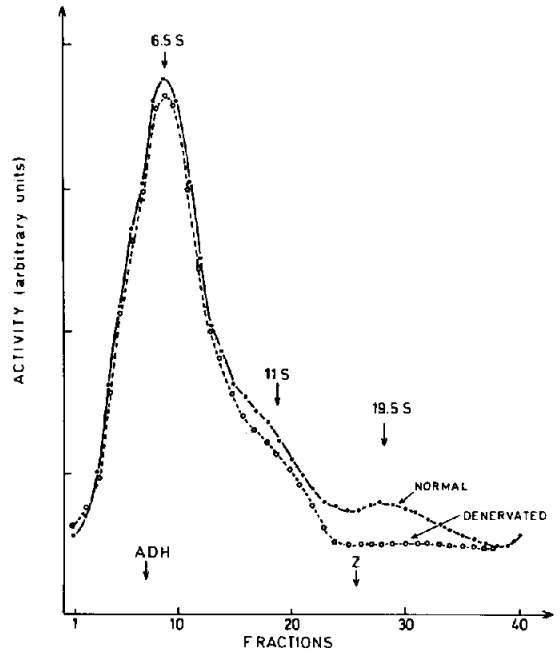

Fig.3. Sedimentation profile of AChE activity from normal and denervated fast muscle (PLD) extracts. •, normal muscle; ○, denervated muscle (3 weeks after denervation); Z, β-galactosidase; ADH, alcohol dehydrogenase. Slow muscle (ALD) gave very similar patterns before and after denervation.

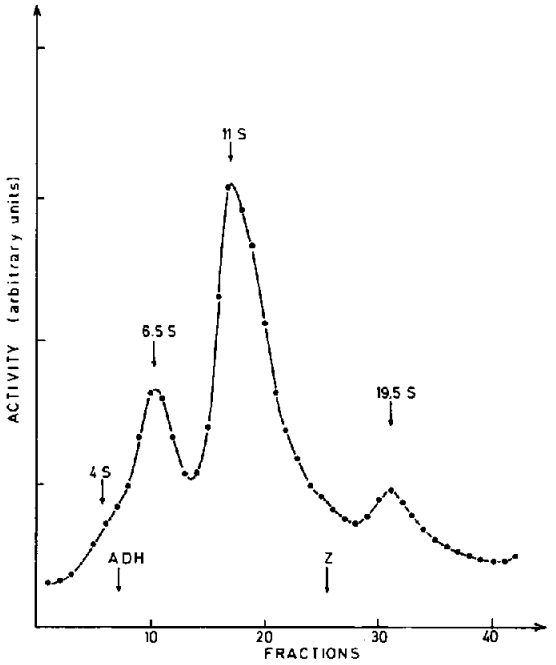

Fig.4. Sedimentation profile of AChE activity from ciliary ganglia extracts. Z, β-galactosidase; ADH, alcohol dehydrogenase.

3.4. *Ciliary ganglia*

Figure 4 shows the sedimentation profiles of AChE from ciliary ganglia extracts. The same forms, 19.5 S, 11 S and 6.5 S described for normal muscles are observed, though their relative proportions is different, plus a small amount of 4 S form. Their percentages are respectively 10, 60, 25 and 5%. The specific activity of ciliary ganglia extract is 6.5 units of AChE per mg of protein (480 nmol AcThCh)/min/mg prot.). The solubilization yield is 400 units per gram of tissue (wet weight).

Detergent-less extraction of ciliary ganglia reduces the solubilization yield to 15–20% of the detergent yield; the three forms are present in the extract, with a majority of 6.5 S.

4. Discussion

Acetylcholinesterase of brain, ciliary ganglia, nerves and muscles (normal and denervated) of young chickens was investigated. Four forms, according to their sedimentation coefficient, were identified. (1) A low molecular weight form of 4 S, which seems to be detectable mostly in peripheral nerves; (2) two intermediate forms, 6.5 S and 11 S, which seems to be ubiquitous; (3) a high molecular weight form, 19.5 S, which is detectable in ganglia and normal muscles and disappears from denervated muscles. None of these four molecular forms of AChE can be considered as an artefact of the Triton X-100 extraction, since they are equally detectable in a detergent-free medium. Triton X-100 has indeed an effect in increasing the extraction yield of the forms 11 S and 19.5 S, probably because of a membranous localization of these two forms.

Finally, the localization of the 19.5 S form in ciliary ganglia and normal muscle, along with its disappearance from denervated muscles, is a further evidence of a strong correlation of this form with cholinergic synapses.

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