DISTRIBUTION OF ACETYLCHOLINESTERASE MOLECULAR FORMS IN NEURAL AND NON-NEURAL SECTIONS OF HUMAN MUSCLE

S. CARSON, S. BON, M. VIGNY, J. MASSOULIÉ and M. FARDEAU

Laboratoire de Neurobiologie Ecole Normale Supérieure 46, rue d'Ulm, 75005 Paris and *Unité INSERM 153, 17, rue du Fer à Moulin, 75005 Paris, France*

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1. Introduction

Acetylcholinesterase can be fractionated, by differential sedimentation in density gradients, into several stable molecular forms [1]. These molecules do not appear to differ in their catalytic activity per active site [2], but correspond to different quaternary associations [3]: in addition to a variable number of active subunits some acetylcholinesterase molecules obtained from *Electrophorus* and *Torpedo* electric organs have been shown to contain a structural rod-like element, or tail [4,5], collagen-like in nature [6,7], which is thought to be involved in the immobilisation of the enzyme within the synaptic cleft [8–10].

Analyses of mammalian acetylcholinesterase have thus far been mostly performed on enzymes from rat tissues [11–14]. Recent experiments performed mainly with bovine superior cervical ganglion enzymes, led us to the conclusion that the various forms of mammalian acetylcholinesterase are structurally similar to those of electric organs [15]. There are three globular forms, which are monomers (G_1, 4 S), dimers (G_2, 6 S) and tetramers (G_4, 10 S) of the catalytic subunits, and three asymmetric tailed form, containing one, two or three tetramers (A_4, 9 S; A_8, 13 S; A_{12}, 16 S). These asymmetric molecules reversibly precipitate in low salt conditions, a property which appears to involve the same type of interactions as the aggregation of the electric organ enzymes.

The heaviest molecule, A_{12}, which is the predominant asymmetric form, has attracted much attention since its location exclusively in the endplate-containing region of the rat diaphragm was shown [11]. This result was confirmed and extended to other rat muscles [13]. After two weeks of denervation this form disappears from the endplate, and reappears at the site of neuromuscular contact when the muscle is reinnervated, either at the original endplate site, or an ectopic site [11,13]. In primary cultures of muscle cells from young rat embryos, the heavy asymmetric form can be induced by the presence of spinal chord explants [16].

The presence of the 16 S form has therefore been considered a biochemical marker for nerve–muscle interactions. If this were the case for human muscle, analysis of acetylcholinesterase might provide a convenient and precise tool for the recognition of potential abnormalities such as occur in various neuromuscular diseases.

We report here an investigation of acetylcholinesterase in normal human muscle in which we compared endplate-containing and endplate-less samples obtained from biopsies of intercostal muscle, removed during surgical operations performed on patients with no neuromuscular abnormalities. A parallel series of experiments performed on biopsies from the deltoid, peroneus or gastrocnemius muscles from patients suffering from various neuromuscular disorders yielded similar results.

Abbreviations: EC 31.1.7 acetylcholinesterase, molecular forms G_1, G_2, G_4, A_4, A_8, A_{12}; the letter indicates the globular or asymmetric nature of the molecule and the subscript its number of catalytic subunits

2. Materials and methods

Small bundles of fibers were prepared from the biopsy specimen, about 8–10 mm long from tendon to tendon, and 0.5 mm wide. These were teased under the stereomicroscope in order to observe the nerve in the medial part of the bundle. The bundle was then separated into two sections, one containing the neural zone (neuromuscular junctions), as well as an aneural segment, and the other containing no endplates. Some fibers were detached from each of these sections and stained by Koelle's method to determine the presence of endplates [17]. The rest of the sections were stored in liquid nitrogen for biochemical analysis. (No difference in sedimentation profiles for a given individual was seen in biopsies used immediately or after storage in liquid nitrogen for up to 2 months.) The fractions (5–20 mg) were finally minced with a razor blade and homogenised with 20 vol. Triton X-100 saline buffer (1 M NaCl, 50 mM $MgCl_2$, 10 mM Tris–HCl, pH 7, 1% Triton X-100) in a Teflon–glass Potter homogenizer. The extracts were analysed immediately after homogeneisation: 200 μl aliquots were layered on 5–20% sucrose gradients (in the same medium) and centrifuged for 21 h at 38 000 rev./min, in a SW 41 Beckman rotor, at 4°C. The following enzymes were included as sedimentation coefficient standard: *E. coli* β-galactosidase (16 S), beef liver catalase (11.3 S) and alcohol dehydrogenase from yeast (7.4 S). Aliquots of the fractions were assayed with the Ellman method as in [12], in the presence of 10 μM ethopropazine (100 μl in 1 ml medium, for ~3 h).

3. Results

3.1. *Molecular forms of human acetylcholinesterase*

Human muscle was found to contain both specific acetylcholinesterase and non-specific cholinesterase. The 'non-specific' component amounted to ~5% of the total cholinesterasic activity. This is markedly less than in rat muscle, where the proportion is 15% [18]. In our analyses, we specifically inhibited the 'non-specific' cholinesterase, and analysed only the molecular forms of the specific acetylcholinesterase, by zone sedimentation in sucrose gradients. Six molecular forms could be identified (fig.1,2) and appeared exactly homologous to those which have been previously characterized in other mammals such as rat and ox. The sedimentation coefficients are essentially identical for the corresponding forms and as reported [15] in the case of ox, rat and chicken, three of these molecules remain soluble in low salt (globular or G forms) whereas three others reversibly precipitate under such conditions (asymmetric or A forms) (table 1).

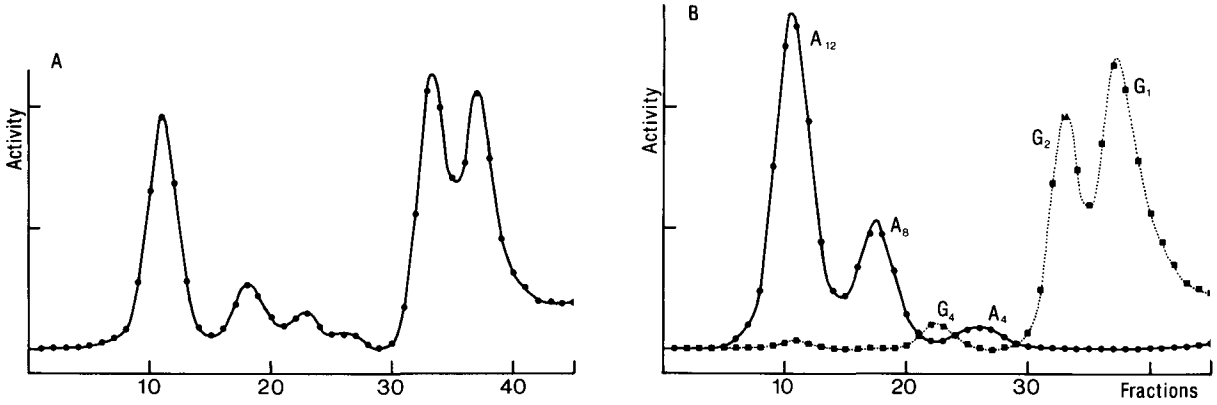

Fig.1. Sedimentation patterns of human acetylcholinesterase. (A) Unfractionated saline extract (1 M NaCl, 0.05 M $MgCl_2$, 0.01 M Tris–HCl, pH 7, 1% Triton X-100). (B) Low salt soluble (■· · · ·■) and insoluble components (●——●). The low salt (0.04 M $MgCl_2$, 0.01 M Tris–HCl, pH 7, 1% Triton X-100) precipitate was redissolved in saline buffer and both components were centrifuged in the same medium. The sucrose gradient (5.2% w/v) were centrifuged as indicated in section 2. Activities are plotted with an arbitrary scale as a function of fraction number. The *A* variation was < 1 unit, i.e., in the linear range.

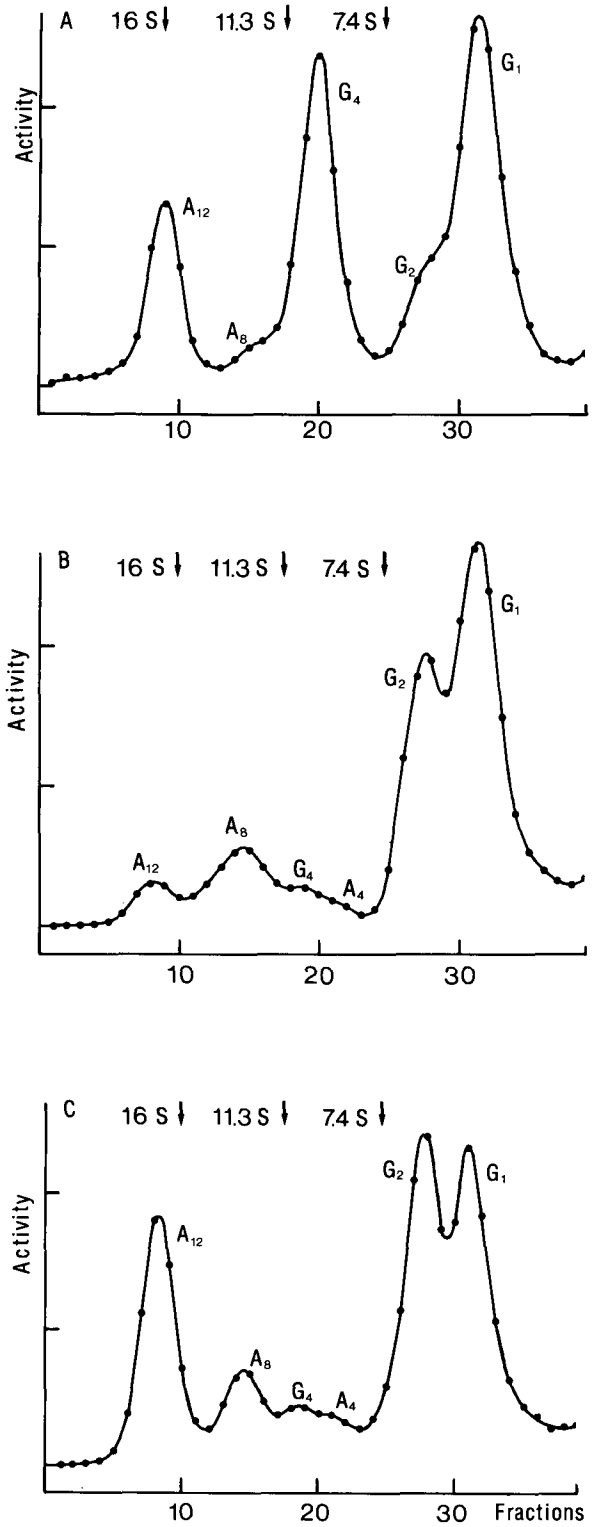

Table 1
Sedimentation coefficient of the acetylcholinesterase molecular forms in human muscle

Globular forms			Asymmetric forms		
G_1	G_2	G_4	A_4	A_8	A_{12}
3.5 S	6 S	11 S	9.5 S	13.3 S	16.7 S

The sedimentation coefficients indicated correspond to the apparent values, obtained under our experimental conditions

In spite of the clear homology between individual forms of acetylcholinesterase, their relative proportions differ markedly in human and rat muscles (see fig.2):

(i) The proportion of the G_2 form in human muscle is much larger than that in rat muscle where it represented only a minor portion of the total acetylcholinesterase activity;

(ii) The proportion of the A_8 (13 S) component is markedly larger than in rat, where its existence has been recognized only recently [15];

(iii) The slower sedimenting asymmetric component (A_4) is readily detectable while it is not in the rat, where it is masked by the G_4 component which is far more abundant ($> 30\%$) than in human extracts ($< 10\%$).

In addition to global species-specific differences, the profiles shown in fig.2 illustrate a very large variability among human individuals. For instance, the heavy A_{12} form was found to vary from $< 10\%$ to nearly 30% of the total activity. There was no obvious correlation between acetylcholinesterase profiles and variables such as age, sex or health condition. These individual differences might be of genetic origin, but this hypothesis should be substantiated by further studies. It is interesting that no such variations have been observed among laboratory rats, but studies with animals of different genetic background have not yet been undertaken.

Fig.2. Sedimentation patterns of rat and human muscle acetylcholinesterase (intercostal muscle extracts). (A) Rat muscle acetylcholinesterase; (B,C) human muscle acetylcholinesterase from two different individuals. The specific activities of the homogenates were 0.35 nmol/h/mg protein in the case of rat intercostal muscle and varied from 0.35–0.6 in the case of human muscle samples.

3.2. *Distribution of molecular forms in innervated and non-innervated sections*

As shown in fig.3, we characterised sections from normal intercostal biopsies from 6 patients with and without endplates and we analysed their acetylcholinesterase molecular forms. In all sections from a given individual, we obtained identical patterns in innervated and non-innervated sections.

The same conclusion was reached when we examined the patterns of endplate-less and endplate-containing sections in 17 pathological cases. There was no correlation therefore between the presence of endplates and the occurrence of any particular molecular form. In particular, the A_{12} (16 S) form was present in all sections with the same relative activity. It is thus clear that this form, which was sometimes called 'endplate form' according to the observations made on various rat muscles [11,13] is not restricted to endplates in human skeletal muscle.

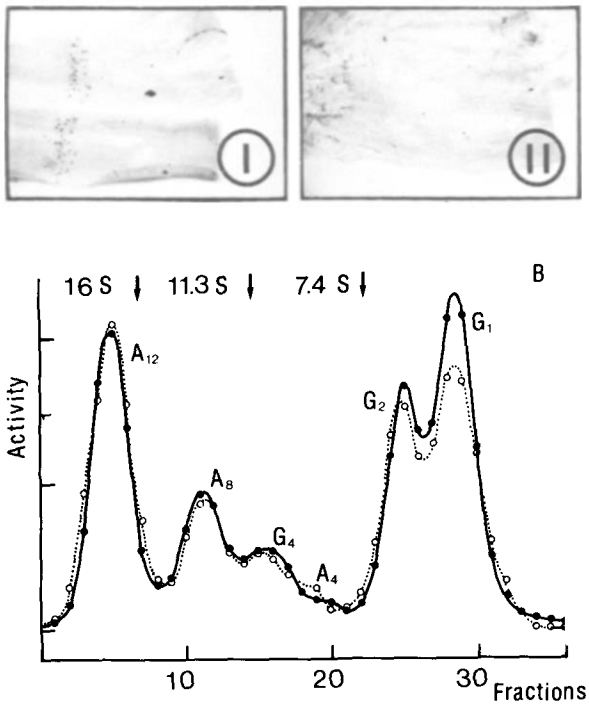

Fig.3. Neural and aneural sections of muscle. (A) Endplates are clearly visible in I and absent in II. (B) Acetylcholinesterase sedimentation profiles of the muscle shown in (A). The two profiles correspond to neural (•——•) and aneural (○– – –○) regions of the muscle; the variation between the 2 profiles is the same as for two profiles of similar regions and reflect the dispersion of experimental results.

4. Discussion

It is very likely that the aggregating properties which are characteristic of the collagen-tailed asymmetric molecules of acetylcholinesterase, allow the binding of this enzyme to polyanionic components of the basal lamina. Such interactions might in particular account for its functional localisation within the synaptic cleft. Such a mechanism appeared remarkably consistent with the endplate localisation of the asymmetric molecules, as observed in rat muscles [11,13]. It is therefore surprising that no such preferential localisation existed in human muscle.

Another similar situation has however been reported [16] where muscles cells from day 18 rat embryos were observed to synthetize A_{12} acetylcholinesterase in absence of neural elements (in contrast with cells from day 13 embryos) and that no patches of esteratic activity appeared on the surface of these cells. It remains however quite possible that the synthesis of the A forms of acetylcholinesterase by muscle cells requires to be triggered by neural induction. Such a regulation might represent a phenomenon quite distinct from an endplate-restricted localisation.

The description [19] of a myasthenic syndrome in which endplate potentials were abnormally long and unresponsive to anticholinesterasic drugs and in which the A_{12} form was missing, was considered in [19] as evidence that this form is indeed subject to a well-defined regulation and might even be the only physiologically active form of acetylcholinesterase in human muscle. We observed, however, very low levels of this molecule and more generally of all asymmetric molecules, in some apparently quite normal individuals.

The two main conclusions of this study are that in mammalian muscles the heavy asymmetric forms are not necessarily localised at the endplate and that their levels are not simply correlated with physiological conditions. A more precise comprehension of the role of the various molecular forms will deserve further study.

Acknowledgements

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