INTERACTIONS BETWEEN LECTINS AND ELECTRIC EEL ACETYLCHOLINESTERASE

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1. Introduction

Multiple forms of acetylcholinesterase, solubilized from *Electrophorus electricus* electric organs have been characterized by their hydrodynamic properties and by electron microscopy observation [1–4]. They all have a higher CsCl isopycnic density (~1.31) than do the other proteins (~1.28), suggesting the presence of some sugar moiety.

Polyacrylamide gel electrophoresis (with SDS and mercaptoethanol) of the purified enzyme has yielded two major protein sub-units, which positively stained with PAS reagent and contained sialic acid residues [5].

The fact that neuraminidase has a pronounced action on the characteristics of the complement fixation of elongated forms of acetylcholinesterase [6] indicates that these sialic acid residues are part of some antigenic determinants of acetylcholinesterase. Furthermore, some results from Rosenberry et al. [7] suggest the presence of a certain amount of carbohydrate on the tetrameric purified form of acetylcholinesterase: amino acid analyses gave a content of 3–4% of hexosamines in this form. Ciliv and Ozand [8], working on erythrocyte acetylcholinesterase, made observations which also establish the presence of carbohydrates, in particular demonstrating that neuraminidase treatment changes electrophoretic mobility of acetylcholinesterase isozymes. Some more details have been given by Taylor et al. [9] on the presence of carbohydrates in *Torpedo* acetylcholinesterase: the total content of carbohydrates is 7.9% including hexoses, hexosamines and sialic acid, in a proteolysed 11 S form.

This paper describes new evidence concerning the nature of the carbohydrate content of elongated forms of electric eel acetylcholinesterase, as suggested by the precipitation of the enzyme by soluble lectins or their attachment onto polyacrylamide beads with gluteraldehyde coupled lectins. We confirm these results by the direct determination of carbohydrate composition using gas chromatography.

2. Materials and methods

Acetylcholinesterase has been solubilized from electric organs of *Electrophorus electricus* and purified following the method recently described [3].

Experiments on acetylcholinesterase–lectin interactions were usually done with the high density acetylcholinesterase isolated by cesium chloride equilibrium centrifugation (specific activity*: 10 000) or with the major activity peak, eluted from the agarose A 15 m column, which contains a mixture of the elongated forms (mostly D with a specific activity: 30 000).

Concanavalin A is from Miles Yeda. Other lectins have been isolated using specific adsorption on glutaraldehyde insolubilized rabbit erythrocytes, followed by elution with 0.2 M glycine, HCl, pH 2.8 [10]. They were insolubilized by glutaraldehyde [10] onto polyacrylamide P 300 beads. Their characteristics are described in table 1. Concanavalin A-Sepharose is a product of Pharmacia.

Experiments of acetylcholinesterase activity precipitation by the soluble lectins were performed in the following manner: 1000 units of acetylcholinesterase were incubated in 1 ml of 1 M NaCl 0.05 M $MgCl_2$

* Specific activities are expressed by optical densities variations per minute at 412 nm in Ellman's medium per optical density unit at 280 nm [1].

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Table 1

	Mol. wts*	Sub units*	Number of binding sites*	Specificity**	Proteolytic*** contamination
Canavalia Ensiformis (Jack Bean)	55 000 (pH≤5.6) 110 000 (5.6 pH 7)	2 4	2 4	α-D-Mannopyranose (αD Man) α-D-Glucopyranose (αD Glc) D-Fructofuranose and their glycosides	1
Lens Culinaris (Lentil)	42 000	2	2	α-D-Glucopyranose α-D-Mannopyranose and their α-Methyl glycosides	8.6
Ricinus Communis (Castor Bean)	98 000			For RCA_{20} and $_{60}$: D-Galactose and disaccharides linked with D Gal at non-reducing end and also, for RCA_{60} NAc D Galactosamine	5.8
Triticum Vulgaris (Wheat Germ)	35 000 [12]	2	4 2 per subunit	(D Glc NAc)1,2,3 etc. and D Glc with 2-acetamido group and 3-OH free group	–
Phaseolus Vulgaris (Red Kidney bean)	150 000 [13]	4		(D Gal NAc)	4,4

* Essentially from [11] unless otherwise indicated.
** And structurally related saccharides
*** Proteolytic activity of lectins (250 μg/ml) expressed as percent of the proteolytic activity of a solution of pure trypsin (10^{-3} mg/ml) able to convert, in 24 hr, the amount of acetylcholinesterase used here, in the globular active form G.

0.01 M Tris pH 7, at 4°C for 72 hr, in the presence of variable quantities of each lectin (usually 0 to 1000 μg per ml). After centrifugation in a Sorvall centrifuge (Rotor SS 34, 20 000 *g*, 30 min), the acetylcholinesterase activity of the supernatants was measured. To study the interactions of acetylcholinesterase with the insolubilized lectins, tubes containing the mixtures of insolubilized lectin and acetylcholinesterase, were gently rocked at 4°C and centrifuged at variable time. The percentage of un-bound activity was then measured in the supernatant.

3. Results and discussion

Lectins are known to precipitate glycoproteins in solutions, by associating with specific polysaccharidic sites on the glycan part of these proteins. This precipitation depends on the accessibility of the glycoprotein site for the lectin and on the presence on the lectin used of at least two binding sites. The precipitation curves of acetylcholinesterase activity for six different lectins are given in fig.1. They show different precipitation efficiencies for a constant amount of acetylcholinesterase. Acetylcholinesterase does not precipitate if the incubation medium contains the free sugar which shows affinity for the lectin (fig.2). None of the lectins used inhibited acetylcholinesterase activity, since homogeneisation of precipitates in buffer generally resulted in satisfactorily homogenous suspensions, with nearly total recovery of acetylcholinesterase activity. We find that lectins, specific for saccharides of the Man type (*Canavalia Ensiformis* and *Lens*

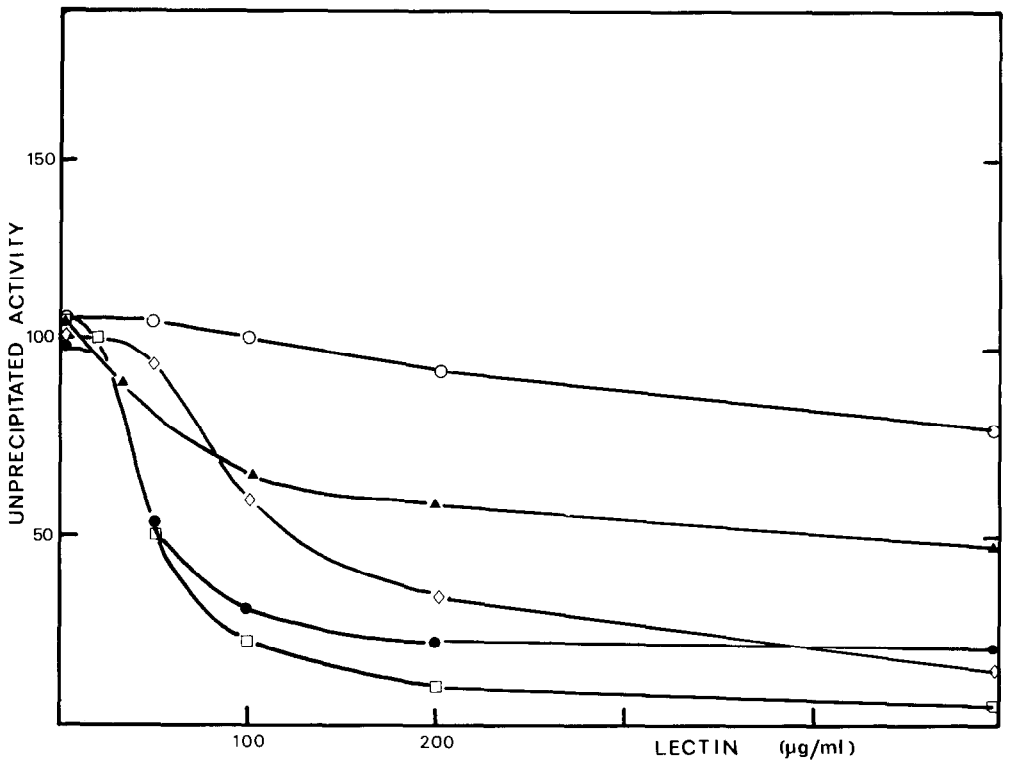

Fig.1. Precipitation curves of acetylcholinesterase by five different lectins: (□–□) *Lens Culinaris.* (●–●) *Canavalia Ensiformis.* (◇–◇) *Phaseolus Vulgaris.* (▲–▲) *Ricinus Communis.* (○–○) *Triticum Vulgaris.* Supernatant acetylcholinesterase activity (in ordinate) is expressed in per cent of the total amount of activity, incubated at 4°C during 72 hr, in presence of 1 M NaCl, 0.05 M $MgCl_2$, 0.01 M Tris pH 7, in function of variable quantities of each lectin. The specific activity of acetylcholinesterase was 10 000.

Culinaris) and D Gal Nac type* (*Phaseolus Vulgaris*) are more effective than lectins specific for galactose (*Ricinus Communis)* and D Glc N Ac (*Triticum Vulgaris*). This difference is perhaps due to the accessibility of each residue (which may depend on its more or less internal localization in the polysaccharide chains). It may also reflect a greater abundance of Man and Gal N Ac residues and/or a higher affinity of these residues for the lectins.

Another piece of evidence for the acetylcholinesterase–lectins association is the detection of a shift in the sedimentation constant of acetylcholinesterase (continuous sucrose gradient ultracentrifugation). This observation is very difficult for two reasons: a) lectin preparations contain some proteolytic activity which causes conversion of the acetylcholinesterase molecular forms (2), (3). b) discrete complexes are not easily obtained because of the rapid formation of heavy aggregates. Nevertheless, we have obtained interpretable results with Concanavalin A, using an incubation medium giving homogenous populations of dimeric molecules of the lectin (pH 5.5: mol wt. 54 000). We have observed an increase of approximately 5 S of the sedimentation constant of the D form.

Acetylcholinesterase is adsorbed on gels coupled to these different lectins. With incubation times of three hours, the specific sugar inhibits about sixty per cent of the control binding. A good retention capacity has been observed with *Canavalia Ensiformis, Lens Culinaris* and *Triticum Vulgaris* lectins, but also with *Ricinus Communis* lectin, which demonstrates a low precipi-

Abbreviations: SDS: sodium dodecyl sulfate. PAS reagent: periodic acid Schiff reagent. αMan: α-Mannose. αGal: α-Galactose. Glc: Glucose D-Gal Nac: *N*-Acetyl D-galactosamine. D-Glc Nac: *N*-Acetyl D-glucosamine. Con. A: Concanavalin A.

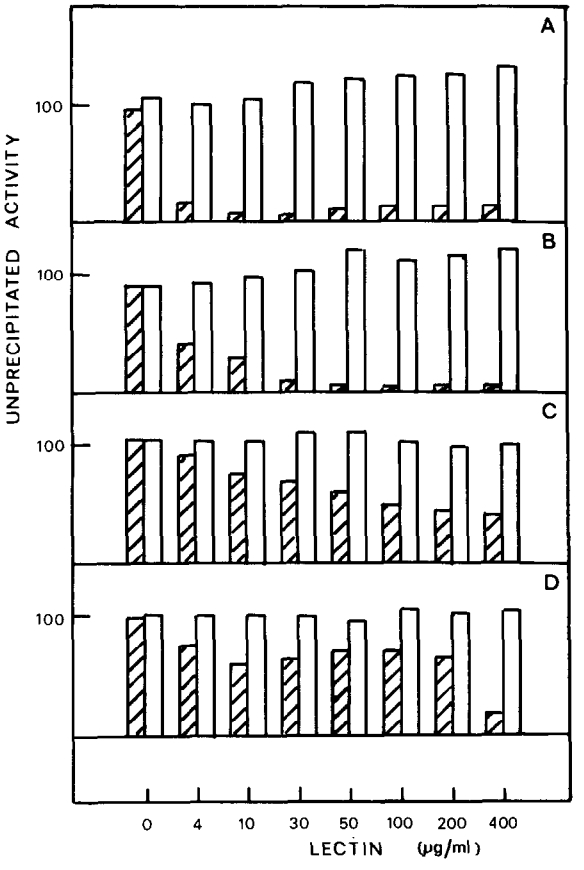

Fig.2. Inhibition of the precipitation of acetylcholinesterase by lectins in the presence of the known specific sugar (empty area). A) *Canavalia Ensiformis* ∓ α Me D Mannoside. B) *Lens Culinaris* ∓ α Me D Mannoside. C) *Ricinus Communis* ∓ D Galactose. D) *Phaseolus Vulgaris* ∓ D Galactosamine. The specific activity of acetylcholinesterase was 30 000. Acetylcholinesterase activity (ordinate) is expressed as in fig.1 and incubations were done in the same conditions. In absence of lectin, but in presence of the sugar, no particular change in activity was observed.

tation efficiency. If a longer incubation time is used (three days), acetylcholinesterase is adsorbed even in the presence of the specific sugar. We do not know whether this effect is due to non-specific binding or to multiple successive binding of several molecules of lectin with the same molecule of acetylcholinesterase.

We have tried to exploit the interaction properties of acetylcholinesterase with lectins in order to purify the enzyme, using Sepharose-insolubilized Con A (8 mg Con A/ml centrifuged beads). We obtain a high adsorption capacity (5000 units per ml centrifuged beads) but many other proteins or glycoproteins are also adsorbed. Besides it is very difficult to elute acetylcholinesterase: even with 0.2 M of αD-mannose plus 0.2 M αD-glucose we recovered only 30% of the total enzyme. Therefore, we do not use this procedure for the purification of acetylcholinesterase.

We confirmed the above analytical data on the purified D form (18.4 S) of acetylcholinesterase by gas chromatography; we have identified, without ambiguity, D-mannose, D-galactose, *N*-acetylglucosamine, and N-acetyl-neuraminic acid. In addition, chromatograms appear to be complex and suggest the presence of some unidentified sugars or lipids or glycolipids, as already indirectly evidenced by the action of pure phospholipase C on the structure and aggregation properties of elongated forms of acetylcholinesterase [14].

We can therefore conclude that acetylcholinesterase has, in an exterior position, some saccharidic moieties, structurally related or identical to α-mannose and/or glucose, galactose, *N*-acetyl glucosamine, *N*-acetyl galactosamine and sialic acid.

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References

[1] Massoulié, J., Rieger, F. and Bon, S. (1971) Eur. J. Biochem. 21, 542–551.
[2] Rieger, F., Bon, S. and Massoulié, J. (1972) C.R. Acad. Sci. Paris 214, 1753–1756.
[3] Rieger, F., Bon, S., Massoulié, J. and Cartaud, J. (1973) Eur. J. Biochem. 34, 539–547.
[4] Bon, S., Rieger, F. and Massoulié, J. (1973) Eur. J. Biochem. 35, 372–379.
[5] Powell, J. T., Bon, S., Rieger, F. and Massoulié, J. (1973) FEBS Lett. 32, 17–22.
[6] Rieger, F., Benda, P., Bauman, A. and Rossier, J. (1973) FEBS Lett. 32, 62–67.

[7] Rosenberry, T. L., Chang, H. W. and Chen, Y. T. (1972) J. Biol. Chem. 247, 1555–1565.
[8] Ciliv, G. and Ozand, P. T. (1972) Biochim. Biophys. Acta 284, 136–156.
[9] Taylor, P., Jones, J. W. and Jacobs, N. M. (1974) Mol. Pharmacol. 10, 78–92.
[10] Avrameas, S. and Guilbert, B. (1971) Biochimie 53, 603–614.
[11] Lis, H. and Sharon, N. (1973) Ann. Rev. Biochem. 42, 541–574.
[12] Nagata, Y. and Burger, M. M. (1974) J. Biol. Chem. 249, 3116–3122.
[13] Weber, T. H., Aro, H., Nordman, C. T. (1972) Biochim. Biophys. Acta 263, 94–105.
[14] Rieger, F., Bon, S. and Massoulié, J. (1973) FEBS Lett. 36, 12–15.