BINDING OF ACETYLCHOLINESTERASES TO CONCANAVALIN A

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1. Introduction

Acetylcholinesterases from a number of different sources are known to be glycoproteins. Carbohydrate residues were found in the eel enzyme [1,2], human erythrocyte acetylcholinesterase [3] torpedo californica [4] plaice cholinesterase [5] and the cholinesterases from human serum [6,7], horse serum [8,9] and human brain [10]. It was of interest to us to examine whether the presence of carbohydrate residues would yield a specific interaction between the plant lectin concanavalin A (con-A) and acetylcholinesterases. Such specific interactions were recently shown with α-antitrypsin [11], β-galactosidase [12] and dopaine-β-hydroxylase [13]. This paper presents evidence that acetylcholinesterases from human erythrocytes, the electric organ of *Electrophorus electricus* and from plaice body muscle are bound to columns of con-A-sepharose and are selectively eluted by α-methyl-D-mannoside. The complex formed between acetylcholinesterase and con-A could be demonstrated by gel filtration on sepharose 4B.

2. Materials and methods

Acetylcholinesterase was extracted from frozen electric tissue of *Electrophorus electricus* by a modified procedure of Hopff et al. [14,15]. The tissue was homogenized in a Virtis overhead homogenisator in a buffer of 10 mM Na phosphate pH 7.4 containing 1 M NaCl. After centrifugation for 20 min at 20 000 *g* the supernatant was treated for one minute with ice cold toluene by low speed blending in the Virtis homogenisator. After centrifugation at 7000 *g* for 30 min and removal of the toluene by aspiration, the 'quark' was separated from the aqueous phase which was reextracted twice with toluene. The combined quark-phases were reextracted with the above buffer. The combined aqueous extracts were centrifuged again for 30 min at 20 000 *g*. They contained 87% of the total acetylcholinesterase activity. Sucrose density gradient centrifugation showed that by this treatment only the elongated forms A, C and D of the enzyme were obtained. Form G, the proteolytic breakdown product of the elongated forms, could not be detected.

As source of form G, the commercially available acetylcholinesterase from electric eel (Sigma Type VI) was used. Human erythrocyte acetylcholinesterase was prepared as described previously [16] and the plaice enzyme was a gift of Dr S. J. Lundin.

Con-A, con-A-sepharose and sepharose 4B were obtained from Pharmacia (Sweden). α-Methyl-D-mannoside, acetylthiocholine and 5,5-dithio – nitrobenzoic acid were purchased from Fluka. All other chemicals were reagent grade. Acetylcholinesterase activity was determined at 30°C by following the production of thiocholine according to the method of Ellman et al. [17].

3. Results

Purified acetylcholinesterases from the electric eel and the plaice, when passed through a column of con-A-sepharose, were adsorbed onto the insoluble support. The enzymes could be eluted by a solution of 10% α-methyl-D-mannoside (fig. 1A,B). The enzyme from human erythrocyte, at first did not bind to con-A-sepharose. As this preparation contained 0.1% Triton X-100 the inability to bind to the insoluble support was attributed to the presence of this detergent. After

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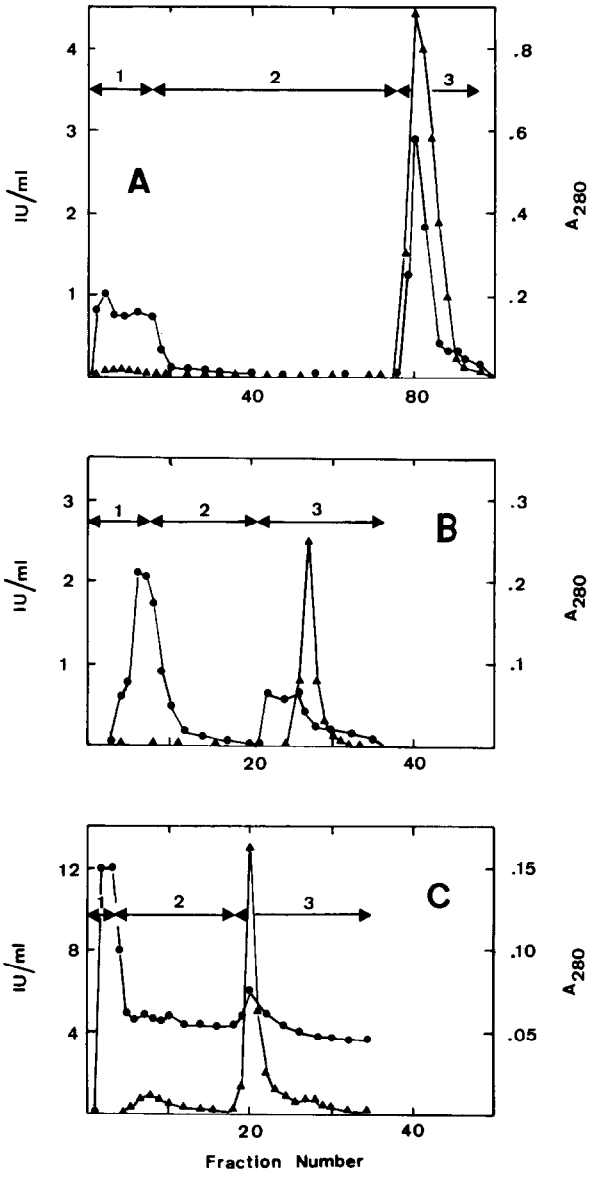

Fig. 1. Chromatography of acetylcholinesterases on concanavaline-A-sepharose. Con-A-sepharose (1 ml) was filled into a Pasteur pipette and equilibrated with a solution of 10 mM Na phosphate, pH 7.0, containing 1 M NaCl. Acetylcholinesterase in the same buffer was applied to the column at a rate of 1 ml per hour (←1→). The column was washed with buffer (←2→) and thereafter the enzyme was eluted with a 10% solution of α-methyl-D-mannoside in the above buffer (←3→). Protein was determined spectrophotometrically at 280 nm (●–●–●) and enzyme activity was measured according to Ellman's method (▲–▲–▲). A: Acetylcholinesterase from the electric organs of the electric eel; B: from plaice body muscle and C: from human erythrocytes.

removing the excess triton X-100 the enzyme was quantitatively adsorbed on con-A-sepharose and could be eluted with α-methyl-D-mannoside (fig. 1C). The recoveries in enzyme activity exceeded 75% in all cases. When the supernatant solutions of toluene-treated homogenates of eel tissue were chromatographed through con-A-sepharose the enzyme initially was bound but due to the presence of other carbohydrate containing protein the column quickly became saturated. When, however, the batch procedure was used all the enzyme could be adsorbed onto the affinity gel. Although a 10-fold purification resulted, this method of enzyme purification was inferior to the anionic center specific affinity chromatography originally described by Berman and Young [18].

According to Massoulié and coworkers [2] the subunits of the eel acetylcholinesterases differ with respect to their carbohydrate content. The separation of the forms A, C and D from G was therefore attempted by chromatography on con-A-sepharose. Neither stepwise elution or elution by a linear gradient of α-

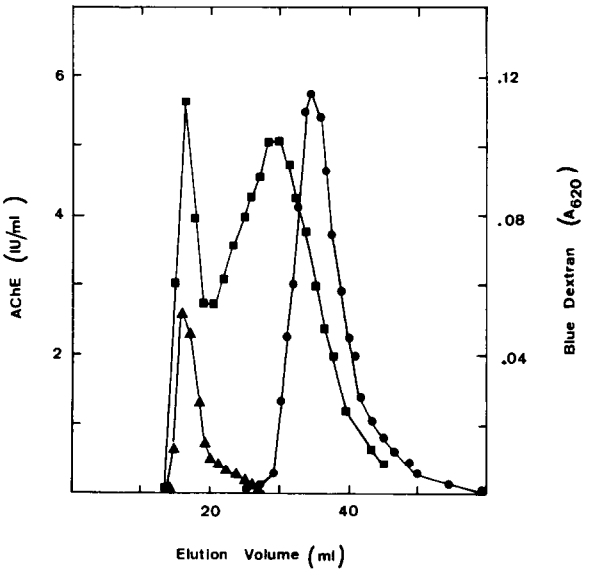

Fig. 2. Gel filtration on sepharose 4B of acetylcholinesterase in presence and absence of con-A. Form G of eel acetylcholinesterase (75 IU) were passed through a column (0.9 × 63 cm) of sepharose 4B equilibrated and eluted with 20 mM Na acetate buffer pH 6.0 containing 1 mM $MnCl_2$, 1 mM $CaCl_2$ and 1 M NaCl. Fractions, 1 ml each, were collected. (●–●–●) Acetylcholinesterase activity in absence and, (▲▲▲) in presence of 0.5 mg con-A. (■–■–■) blue dextran.

methyl-D-mannoside proved to be effective in separating the elongated forms of the enzyme from the more globular one. When assayed in presence of increasing amounts of con-A (up to 10 μg/ml) no decrease in enzyme activity could be observed. Prolonged incubation at 4°C however caused a 30% drop in activity. Addition of 1% α-methyl-D-mannoside restored acetylcholinesterase activity to 93% of the control value (measured in absence of con-A). The formation of a complex between con-A and acetylcholinesterase could be demonstrated by chromatography on sepharose 4B (fig. 2). Form G of the eel enzyme in absence of con-A was eluted in a volume of 34 ml. The elution volume decreased to 16 ml, the void volume of the column, in presence of con-A. When the column was preequilibrated with buffer containing 1% of α-methyl-D-mannoside, no complex was formed between the enzyme and the lectin; the enzyme activity eluted with the same volume as the one obtained in absence of con-A.

4. Discussion

Besides the recent report of Taylor and coworkers [4] on the acetylcholinesterase from *Torpedo california*, to our knowledge no other evidence exists for the specific interaction of a plant lectin with acetylcholinesterases. The fact that the enzyme from electric eel, plaice body muscle as well as from human erythrocytes interacts with con-A-sepharose adds additional evidence for the glycoprotein nature of these enzymes. As suggested by Massoulié [19] the tail-like position of the eel enzyme might be responsible for its attachement to the membrane. It was suggested that the tail portion contains considerable amount of carbohydrate residues. The results presented in this paper support this hypothesis in as much as con-A binds to acetylcholinesterase without impairing its enzymatic activity. As suggested by Taylor et al. [4] the hydrophilic oligosaccharides may function to orient the enzyme relative to the membrane surface.

The molecular weight of form G of acetylcholinesterase is 260 000 [1]. Con-A at pH 6 is a dimer with a molecular weight of 55 000 [19]. As the complex formed between these two proteins eluted with the void volume of the sepharose 4B column, its molecular weight exceeded $2 \cdot 10^6$ indicating the formation of either large aggregates between the enzyme and con-A or a highly asymetric complex resembling the elongated forms of acetylcholinesterase [19].

It is of interest to note that Triton X-100 solubilized human erythrocyte acetylcholinesterase in presence of this detergent did not adhere to con-A-sepharose. This detergent probably shielded the carbohydrate moieties of the enzyme from interacting with the plant lectin. Only after removal of excess Triton X-100 became the carbohydrate binding sites available to the interaction with con-A.

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