PURIFICATION OF CHOLINESTERASE FROM OX RED CELLS

by

J. A. COHEN AND MIA G. P. J. WARRINGA

Medical Biological Institute of the National Defence Research Council T.N.O., Leyden (Netherlands)

True cholinesterase has been the subject of intensive study, owing to the important role played by this enzyme in animal physiology. These studies have been hampered because of the unavailability of methods to produce reasonably pure soluble preparations of mammalian cholinesterase. Methods described in the literature[1-6] are unsatisfactory for various reasons (*e.g.* poor solubility or purity, irreproducibility).

A method is reported for preparing a soluble cholinesterase preparation from ox red cells, which represents a 250–400 fold purification as compared with the starting material.

Analytical methods

The assays of cholinesterase were carried out by AMMON's manometric method[7] adding 5 mg of acetylcholine in 0.25 ml from the side arm to give, with bicarbonate Krebs Henseleit Ringer in the main vessel, a final volume of 3 ml. The atmosphere consisted of N_2 + 5% CO_2 and the bath temperature was 37° C. One unit of cholinesterase is defined as that amount of enzyme which, under the above conditions, produces one ml of carbon dioxide per hour.

Nitrogen was estimated using either the KJELDAHL or DEKKER's method[8]. Where ammonium sulphate was present protein was first precipitated with one volume of 20% trichloroacetic acid and washed three times with 10% trichloroacetic acid. (Centrifugation in Servall superspeed vacuum centrifuge SS_2 at 14,000 r.p.m.) The washed precipitate was taken up in a small volume of molar NaOH and tested.

Procedure

3 l defibrinated ox blood from freshly slaughtered animals are centrifuged during one hour at 0° C. The serum and the upper layer are discarded and the red cells are washed six times with 0.9% NaCl. The cells are hemolyzed overnight in 10 to 15 volumes of distilled water after addition of a few lumps of solid CO_2 (I). The stroma settles down, is washed five times with a Tyrode solution diluted 1/9, taken up in approximately 1 l glass-distilled water (II) and divided over two to three bottles to be freeze-dried*. The freeze-dried material is suspended in approximately 1 l dry cold butanol, mixed for one minute in the Waring blender and left for 10 minutes in the cold. The material is centrifuged for 15 minutes at 2000 r.p.m. and the supernatant discarded. All manipulations so far described take place in the cold room or the cooled centrifuge.

The precipitate is dried in an evacuated desiccator in the presence of silica for several hours. The dried material (approx. 7 g) is now taken up in 0.01 molar phosphate buffer at pH 8.0 (1 g per 100 ml). The suspension is treated for one minute in the Waring blender and then centrifuged in the Servall superspeed vacuum centrifuge SS_2 at 14,000 r.p.m.; all the centrifugations still to be described were carried out similarly. The supernatant (III), approx. 500 ml, is treated with an equal volume cold saturated ammonium sulphate (AS) at pH 7.0 and kept in the refrigerator overnight. After precipitation the sediment is taken up in 250 ml cold glass-distilled water (IV), 69.5 ml of cold saturated AS are added and the pH is adjusted to 6.0. A small precipitate is formed after stirring and standing for 30 minutes at 0° C; this is spun down. To the supernatant is added 280 ml of saturated AS at pH 6.0. The precipitate obtained after two hours standing and spinning is taken up in 2% NaCl in 0.01 *M* phosphate buffer of pH 8.0 (V). The solution is placed in ice and 8 g of Lloyds reagent are added while the pH is kept at 6.0.

The next morning the solution is spun and the supernatant (VI) is treated with 1.8 volume of saturated AS. After standing for several hours in the cold the precipitate is spun off and taken up in about 30 ml of phosphate buffer pH 8.0 and again centrifuged to discard insoluble material (VII). Dialysis against 1% NaCl for 20 hours in the cold room may be applied to remove remaining AS.

To illustrate the course of purification which is effected by the method described, figures are given for a number of fractions (I–VII) of a representative average preparation (Table I). It will be seen from Table I that a 368-fold purification is achieved.

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TABLE I

Stage	*Purity**	*Times purification*
Hemolysate (I)	101	—
Stroma (II)	2,070	20.5
Buffer extract (III)	2,460	24.3
1st AS prec. (IV)	10,100	100.0
3rd AS prec. (V)	8,800	87.2
After Lloyds reagent (VI)	12,000	119.0
Final preparation (VII)	37,200	368.0

* The figures express purity in units activity per mg nitrogen

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SÉPARATION PAR PRÉCIPITATION ACÉTONIQUE DES DEUX CONSTITUANTS DE LA LACTÉNINE

par

J. E. AUCLAIR*

Station Centrale de Microbiologie et Recherches Laitières, Institut National de la Recherche Agronomique, Paris (France)

ET

N. J. BERRIDGE

National Institute for Research in Dairying, University of Reading (England)

Le terme de "lacténine" a été donné par JONES ET SIMMS[1] à une substance existant dans le lait cru, responsable de l'inhibition exercée par ce lait cru sur divers microorganismes et en particulier sur les streptocoques hémolytiques. JONES ET SIMMS montrèrent que cette substance, non dialysable et thermolabile, est précipitée avec les protéines du lait. Ils obtinrent une préparation concentrée de lacténine par digestion trypsique de lactosérum, suivie de dialyse et concentration.

Dans une publication récente[2], nous avons montré que l'inhibition de *Streptococcus pyogenes* par le lait cru est due en réalité à deux substances distinctes agissant en association, lacténine 1 (L_1) et lacténine 2 (L_2). La teneur du lait en ces deux substances dépend essentiellement du stade de lactation de la vache; le colostrum se montre particulièrement riche en L_1 tandis que L_2 se trouve normalement dans le lait de vache en cours de lactation. Des méthodes ont été décrites pour le dosage de L_1 et L_2 séparément.

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