ACETYLCHOLINESTERASE FROM HUMAN ERYTHROCYTE MEMBRANES: DIMERS AS FUNCTIONAL UNITS

Peter OTT, Ariel LUSTIG*, Urs BRODBECK and Jürg P. ROSENBUSCH*

Medizinisch-chemisches Institut der Universität, PO Box, 3000 Berne and **Biozentrum der Universität Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland*

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1. Introduction

Purified human erythrocyte membrane acetylcholinesterase exists as homogenous enzyme species in the presence of detergents. M_r estimations of the solubilized enzyme have yielded values from 66 000–$\geqslant$362 000 [1–3]. Most of these analyses have been hampered by the fact that hydrodynamic methods, such as density gradient centrifugation and gel filtration were used without quantitation of protein-bound detergent. Alternatively, detergent-depleted enzyme forms were used in investigations to elucidate the M_r-values of the resulting multiple aggregates. In these analytical ultracentrifugation studies, 500 000–1 137 000 M_r has been observed [4]. Yet, no reliable value for the M_r of the acetylcholinesterase protomer has been made available.

This communication describes the estimation, by a thermodynamic method, of the mass of the enzyme in presence of a detergent which does not affect enzymatic activity and which permits M_r determinations without measuring the amount of detergent bound to the protein molecules. The results show that human erythrocyte acetylcholinesterase in detergent solution exists in a monodisperse state with M_r 151 000 ± 8000.

2. Materials and methods

Erythrocyte membranes, prepared according to [5] were used as starting material for acetylcholinesterase purification. The enzyme was solubilized with 10 mM Na-phosphate buffer (pH 7.4) which contained 1% Triton X-100 and 0.05% NaN_3. The solubilized acetylcholinesterase was purified by 2 subsequent affinity chromatography steps, essentially as in [6]. Triton X-100 was removed from the enzyme preparation by chromatography on hydroxylapatite (Bio-Gel HTP), and the protein eluted from the column with a 20 mM sodium phosphate buffer (pH 7.4) containing 0.1 M NaCl, 3 mM NaN_3, 0.2 mM EDTA and 1% octyl-tetraoxyethylene. For analytical ultracentrifugation, purified enzyme was extensively dialyzed against this same buffer. The partial specific volume of micelles of this detergent is 1.005 cm^3/g, so that disregarding its contribution causes an error of ~1%. The rationale for the method used and the detailed procedure have been described in [7].

Ultracentrifugation was carried out on a Beckman model E analytical ultracentrifuge equipped with a photoelectric scanning absorption system. Sedimentation equilibrium experiments were performed at 6 different protein concentrations (0.15–0.35 mg/ml initial conc.) and were monitored using an AN-G rotor equipped with a multiplexer. Rotor speeds were 6000, 11 000 or 12 000 rev./min. The partial specific volume of the enzyme (0.755 cm^3/g), used for M_r calculations, was determined in [4]. Sedimentation coefficients obtained at 56 000 rev./min were corrected to standard conditions using established procedures [8]. Electrophoretic mobilities of acetylcholinesterase in sodium dodecyl sulfate (SDS) on polyacrylamide gels and enzymatic activities were assessed before and after ultracentrifugation. No significant alterations of the enzyme during the experiments was observed.

Enzyme activity was assayed by the procedure in [9], with either 0.1% Triton X-100 or octyl-tetraoxyethylene included in both, assay medium and protein-free control. To determine the number of erythrocytes in cell suspensions a Coulter Counter S plus was used.

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3. Results

Purification of human erythrocyte acetylcholinesterase by 2 successive affinity chromatography steps resulted in spec. act. 5700 IU/mg protein, which is some 40% higher than reported in [1,10]. No loss of activity was observed when the enzyme was dialyzed against the buffer, containing the detergent octyl-tetraoxyethylene, used for analytical ultracentrifugation.

The M_r of this preparation, determined by sedimentation equilibrium, was 151 000 ± 8000. Fig.1 shows little, if any, deviation from straight lines, a result observed in all experiments performed. Determination of the subunit M_r by SDS–polyacrylamide gel electrophoresis gave apparent subunit M_r 73 000. Sedimentation velocity experiments yielded 5.7 ± 0.2 S for the protein–detergent complex. No indication for heterogeneity could be found in these experiments either.

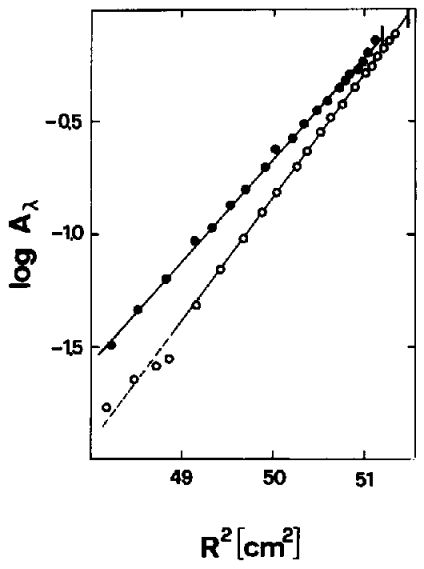

Fig.1. Sedimentation equilibrium analysis of acetylcholinesterase. Centrifugation was done as in section 2. Closed symbols, experiment performed at 15.5°C and 12 000 rev./min and 0.15 mg protein/ml initial conc. Absorbance was monitored at 280 nm. Open symbols, experiment performed at 15.2°C and 11 000 rev./min and 0.23 mg protein/ml initial conc. Absorbance was monitored at 290 nm. The position of the lower menisci in the 2 expt. are indicated by vertical lines (top-right).

4. Discussion

The results of sedimentation equilibrium centrifugations yielded 151 000 ± 8000 as acetylcholinesterase M_r in a solubilized state. The subunit M_r is 73 000, demonstrating that the enzyme occurs as a dimer in non-denaturing detergent solutions. The linearity of the results observed in fig.1 and the homogeneity of the preparation documented in sedimentation velocity experiments indicate that these dimers show no tendency to aggregate over the concentration range investigated (0.01–0.4 mg/ml at equilibrium). The detergent thus provides the environment necessary to stabilize the dimeric enzyme species, which readily aggregates in the absence of detergents at >2.5 μg/ml [11]. The sedimentation coefficient determined in octyl-tetraoxyethylene cannot be directly compared to the *s*-value obtained by sucrose density gradient centrifugation in Triton X-100 (6.3 S, [10]). Yet, the homogeneity of the enzyme in both detergents, and the correspondence of the observed values, makes it probable that acetylcholinesterase exists as a dimer in either detergent, a result consistent with those obtained from crosslinking studies using Triton-solubilized acetylcholinesterase [12]. It should be noted that these methods do not permit the calculation of the protein frictional ratio from the M_r and the sedimentation coefficient of the protein–detergent complex. Thus, it is not possible to decide whether dimeric human erythrocyte membrane acetylcholinesterase is an asymmetric or a globular enzyme form [13].

The recovery of enzymatic activity from erythrocytes is quantitative in non-ionic detergent solutions (unpublished). From the enzyme activity of red blood cell suspensions and the number of cells, it was possible to calculate that 700–800 acetylcholinesterase dimers are present/red blood cell, which corresponds to ~40 μg enzyme/ml erythrocyte membrane volume [14]. This concentration would be high enough to allow aggregation of enzyme dimers to occur, with their activity preserved by hydrophobic interactions [11]. Activity appears equally well stabilized by other hydrophobic surroundings, such as phospholipids or membrane proteins. Which of these interactions maintains enzyme activity in its native surrounding and promotes the anchoring of the enzyme in the red blood cell membrane remains to be investigated.

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