SOME PROPERTIES OF THE CHOLINACETYLASE OF THE HUMAN PLACENTA

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Some properties of the cholinacetylase of the human placenta were studied. The effect of pH of the medium on activity of the enzyme was investigated and the optimum pH determined. The molecular weight of the enzyme was found by gel filtration. Further purification of the enzyme was carried out by the use of this method.

Acetylcholine transferase (acetyl-CoA:choline-O-acetyltransferase, 2.3.1.6) or cholinacetylase (CA) is an enzyme system which participates in the final stage of acetylcholine biosynthesis by transferring the acetyl residue from acetyl-CoA to choline. This enzyme is found in various tissues in both lower and higher animals. The richest and most convenient source for the isolation of CA is the mature human placenta. The writer has previously [1] described a method of isolation and purification of CA from the placenta and given an account of some of the properties of the enzyme.

This work is a continuation of the study of the properties of CA.

EXPERIMENTAL METHOD

Isolation and purification of CA from the placenta were carried out by the method described earlier [1].

The effect of pH of the medium on activity of the enzyme was studied in a medium containing the following components in micromoles in 1 ml (volume of the sample): choline-HCl 11.5, acetyl-CoA 1.9, KCl 160, $MgCl_2$ 4.9, cysteine-HCl 40, eserine salicylate 0.13, sodium phosphate (or Tris-HCl) 12.8, cholinacetylase 250 μg.

The molecular weight was determined by gel filtration on a column packed with Sephadex G-100, superfine fraction. The size of the column was 1.3× 57 cm (total volume 80 cm^3). The eluting solution was 0.1 M phosphate buffer containing 0.4 M KCl, pH 7.0. The rate of flow of the eluate was 6.5 $ml/cm^2/h$.

EXPERIMENTAL RESULTS

CA activity during investigation of the pH optimum was determined in the presence of synthetic acetyl-CoA as donor of the acetyl residue. Curves showing enzyme activity as a function of pH are given in Fig. 1. The optimum of activity in phosphate buffer is in the region pH 6.5-7.0; in Tris-HCl buffer it is in a narrower interval: 6.8-7.0. CA activity in the region of the pH optimum in Tris-HCl buffer was higher than in phosphate buffer. This fact can probably be attributed to the presence of chlorine ions in the medium, for as Schuberth [7] as shown, these ions increase CA activity.

There is little information on the molecular weight of CA. Bull and Morris [4] and Bull et al. [5] determined the molecular weight of CA from the human placenta and rabbit brain by a sedimentation method in a sucrose density gradient. The enzyme preparations studied by these workers were purified only by

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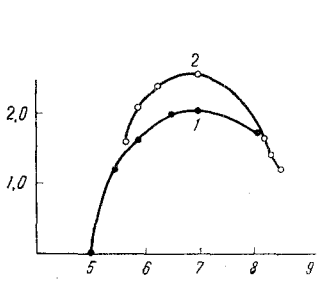

Fig. 1

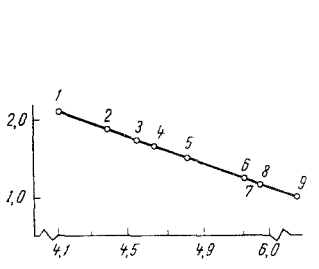

Fig. 2

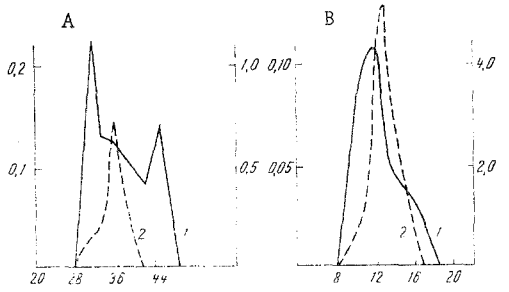

Fig. 3

Fig. 1. Cholinacetylase activity as a function of pH: 1) phosphate buffer; 2) Tris-HCl buffer. Abscissa, pH values; ordinate, enzyme activity (in units/mg protein).

Fig. 2. Calibration curve for determination of molecular weight: 1) ribonuclease; 2) trypsin; 3) pepsin; 4) egg albumin; 5) serum albumin (monomer); 6) serum albumin (dimer); 7) lactate dehydrogenase; 8) alcohol dehydrogenase; 9) blue dextran. Ordinate, ratio between elution volume (v_e/v_0); abscissa, logarithm of molecular weight.

Fig. 3. Diagram of elution of cholinacetylase from a calibrated column (A) and during repeated gel filtration (B): 1) protein content; 2) enzyme activity (in units/ml). Abscissa, elution volume (in ml); ordinate: left) absorption of protein 280 nm, right) CA activity (in units/ml).

fractionation with ammonium sulfate from homogenate of the corresponding tissues. They found that the molecular weight of CA from human placenta and rabbit brain is 59,000 and 67,000 respectively.

In the present investigation the molecular weight of the enzyme preparation isolated and purified by the method described earlier [1] was determined by another method: by gel filtration on a column with Sephadex. The principle of the method of determining the molecular weight of proteins by gel filtration is based on the fact that the elution volume of proteins from the column is a linear function of the logarithm of the molecular weight [2, 3]. To determine molecular weight of a protein, all that is therefore necessary is to find the elution volume of that protein from a previously calibrated column.* To construct a calibration curve, the most widely studied proteins with known molecular weights were used as standards. To determine the molecular weight it was assumed that the logarithm of the molecular weight is a linear function of the ratio between the elution volume of the standard protein (v_e) and the free volume (v_0). The elution volume of blue dextran, with a molecular weight of 2×10^6, was taken as v_0 [6]. Because of the high molecular weight of this compound it can be assumed that its particles do not penetrate into the granules of the gel. The calibration curve is shown in Fig. 2.

The column used (see above) ensured the most complete separation of proteins with the formation of sharply pointed peaks. After application of 4.5 mg CA to the column, elution was carried out with the above-mentioned buffer solution. The enzyme activity and protein content in the eluates were determined from the absorption of light at a wavelength of 280 nm. The elution diagram of CA from the column is shown in Fig. 3A.

As Fig. 3A shows, the enzyme preparation was eluted as three protein peaks: the first protein peak corresponded to a molecular weight of 89,000, the second (the shoulder on the first) to a molecular weight of 59,000, and the third to one of 33,000. Fractions eluted between the first and third protein peaks possessed enzyme activity. It was deduced from the calibration curve that the elution volume of the enzyme (36-36.5 ml) corresponds to a molecular weight of 59,000-58,200. The value obtained agrees with the molecular weight of CA determined by the sedimentation method in a sucrose density gradient [4, 5].

Since only the protein with a molecular weight of 59,000 possessed enzyme activity (as is clear from (Fig. 3A), an attempt was made to purify the enzyme preparation by repeated gel filtration. After the fractionation procedure [1], the CA was subjected to gel filtration on a column measuring 2.5×60 cm ($V_t = 300$ cm^3) filled with Sephadex G-100. The active fractions, after salting out with ammonium sulfate, were subjected to analytical fractionation on a column of the same size as that used to determined the molecular weight. The elution pattern is shown in Fig. 3B.

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Two peaks can be seen on this diagram: one a protein peak, the other a peak of CA activity. Fractions onthe righ-hand slope (shoulder) of the protein peak possessed enzyme activity. The fact that the protein peak does not coincide with the activity peak is evidence of the insufficient degree of purification of the enzyme.

During elution of the protein from the column, an increase in the specific activity of the enzyme was observed. This confirmed earlier findings which showed that the specific activity is increased during fractionation. This may be explained by dissociation of the aggregated complexes formed in concentrated solutions of the enzyme.*

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