Multiple Forms of Choline Acetyltransferase from Rat Brain

We have found that several different forms of choline acetyltransferase (ChAc) from rat brain can be separated by isoelectric focusing. Such heterogeneity of ChAc is of particular interest in the context of its ultrastructural localization. Sub-cellular fractionation¹⁻⁴ and histochemistry⁵ have shown that the enzyme in rat, in conditions of low ionic strength and pH, adhered to several different membranous structures.

Partially purified ChAc from rat brain⁶ was dialysed against 10 mM sodium phosphate buffer, pH 7.4, and electrofocused, using LKB 8101 Ampholine electrofocusing equipment as described before⁷. Carrier ampholytes, pH 3-10 or 6-9, were used in a final average concentration of 1%. The load was 0.5 W for the pH gradient 3-10 and 0.75 W for the pH gradient 6-9. Electrofocusing was carried out at 1° C for 46 h, constant current being obtained after 36 h.

Fractions of 2.5 ml. were collected, the pH was determined with a Radiometer pH meter 22, protein was determined by absorbance at 280 nm and ChAc activity was determined with 1-¹⁴C-acetyl-CoA⁸. Ampholytes did not interfere with the determination of the protein or ChAc.

Results of typical isoelectric focusing experiments are shown in Fig. 1. In the pH gradient 3-10 (Fig. 1a), there was a broad zone or two peaks of enzyme activity between pH 7.7 and pH 8.3. To investigate whether this broad zone corresponded to heterogeneous forms of the enzyme, fractions A and B were collected as illustrated, concentrated by ultrafiltration and refocused in two separate columns with the pH gradient 6-9. The redistribution curves of enzyme activity in fractions A and B did not overlap, so the fractions could not have been derived from a single homogeneous band (Fig. 1b). Fraction A was further separated into two peaks with isoelectric points 7.5 and 7.8 whereas fraction B was confined to a broad zone between 8.2-8.4. When the same enzyme preparation as in Fig. 1a was focused directly in a pH gradient 6-9, three peaks were obtained with isoelectric points 7.5, 7.8 and 8.3.

A similar distribution pattern was obtained when rat ChAc was focused in a medium containing 3 M urea to decrease possible aggregation of proteins or any glass wall effects, or when the focusing time was increased to 72 h.

To exclude the possibility that the heterogeneous forms were a product of enzyme purification, a high speed supernatant of rat brain homogenate was used. Isoelectric focusing of this enzyme gave three distinct peaks of enzyme activity, the largest with an isoelectric point at pH 8.4, the other of pH 7.6 and 7.9. In contrast, isoelectric focusing of purified ChAc from pigeon and guinea-pig brains gave single peaks of enzyme activity with isoelectric points at pH 6.6 and 6.7 respectively. Species differences in isoelectric points agree with the differences found in surface charge⁶.

We suggest therefore that ChAc from rat brain is heterogeneous and consists of at least three closely related forms which may differ in functions and ultrastructural localizations.

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Fig. 1 a, Isoelectric focusing of a partially purified enzyme preparation in a pH gradient 3-10. A sample (13 mg of protein) with specific activity 0.9 μmol of ACh/mg of protein/h was put on two columns. Recovery of ChAc activity (Δ , $\triangle$) was 70-80%. —, pH measurements; $\bullet$ — $\bullet$, A_{280} . b, Refocusing of the pooled fractions A and B in pH gradient 6-9. The fractions were concentrated by ultrafiltration against 10 mM sodium phosphate buffer, pH 7.4, with 5% sucrose before isoelectric focusing. Fraction A consisted of 4 mg of protein, specific activity 1.9 μmol of ACh/mg of protein/h, whereas B contained 0.9 mg, specific activity 3.1 μmol /mg of protein/h. The recoveries were 55 and 50% respectively. c, Isoelectric focusing of the same enzyme preparation as in a but in a pH gradient 6-9. Recovery of enzyme activity was 60%.

⁷ Vesterberg, O., and Svensson, H., *Acta Chem. Scand.*, **20**, 820 (1966).

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