the choline acetyltransferase activity observed was the same as in samples of these tissues prepared by conventional methods (Table I).

We originally had the idea of using cellophane simply as a convenient vehicle for inserting fresh tissue into a small test tube. It was an unexpected piece of good fortune to find that tissue smears and sections were active without further treatment. We had assumed that they would be only as active as untreated homogenates of nervous tissue and were proposing to activate them either by adding a surface-active reagent to the incubate, with dubious chances of success, or by applying ultrasonic vibrations (which effectively activate some preparations) to the incubation medium after the tissue had been immersed in it. As it turned out no further treatment was necessary. However, we do not as yet know by what means the activation of the tissue, on glass or cellophane, or of the homogenates on filter paper is effected.

It seems probable that the important factor in all three methods is that the enzyme is available to its substrates over a larger surface than is provided when homogenized tissue is added to the medium in the usual way. If so, the activating effect of ether may not be due to the release of occult enzyme, as is sometimes assumed, but to an effect on the enzyme-carrying particles which lead to their more even dispersal through the incubation fluid.

To summarize, three methods of preparing small samples of nervous tissue for estimating their choline acetyltransferase are described. The first method, suitable for all types of tissue, is to apply an homogenate in small volume to filter paper, dry it and incubate the impregnated paper in a solution containing the substrates required for acetylcholine synthesis. The second, suitable for samples of up to 2 mg of soft tissues, is to make a smear of the tissue on cellophane and incubate it in the usual way. In the third method, suitable for all types of tissue, fresh-frozen sections replace the tissue smear. With all three methods the choline acetyltransferase activity of the tissue tested was found to be equal to or in excess of the activity of the same tissues prepared by established procedures.

A.R.C. Institute of Animal Physiology, Babraham, Cambridge (Great Britain)

G. BULL
CATHERINE HEBB
DOBRILA RATKOVIC

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Received July 5th, 1962

Purification and properties of phosphoglycerate kinase from chicken breast muscle

SC 11024

Phosphoglycerate kinase (ATP: D-3-phosphoglycerate 1-phosphotransferase, E.C. 2.7.2.3.)activity has been demonstrated in a great variety of animal and vegetal tissues[1]. The enzyme has been crystallized from yeast by BÜCHER[2] and partially purified from pea seeds[3]. The purification and some properties of the rabbit-muscle

enzyme have been recently described by CZOK AND BÜCHER in a preliminary report[4] and by RAO AND OESPER[5].

In the present investigation, an advanced stage of purification of phosphoglycerate kinase from chicken breast muscle has been reached by a rather simple procedure, and some properties of the purified solutions have been investigated. All fractionation procedures were carried out at 2°. The minced muscle was extracted with 1.5 vol. 1 mM EDTA (pH 7.0) for 1 h and centrifuged at 12 000 rev./min. The clear supernatant was brought to pH 8.4 and a $(NH_4)_2SO_4$ solution (pH 8.4) slowly added until 55% saturation was reached. After centrifugation, solid ammonium sulphate was added to the supernatant and the following fractions were successively isolated: 55–65%; 65–70%; 70–95% satn. About 40% of the total initial activity was present in the last fraction, corresponding to a 3.5- to 3.8-fold increase in specific activity.

After dialysis against 1 mM EDTA, the 70–95% satn. fraction was freeze-dried without loss of activity. About 1 g of the dried powder was dissolved in 5 ml barbitone acetate buffer[7] (I 0.05, pH 8.2). Part of the material did not dissolve and the precipitate was centrifuged down. The clear concentrated solution was then fractionated by zone electrophoresis on a 3 cm × 75 cm cellulose bed vertical column[8], in the same buffer at 4°. The field strength applied was about 14 V/cm. After 46 h, the column was eluted with the barbitone acetate buffer at a flow rate of 200 ml/h, 10-ml fractions being collected. Protein and activity distribution in the different fractions are represented in Fig. 1.

The chemical enzymic assay, using hydroxylamine as trapping agent[3] in the incubation medium, has been satisfactorily used after slight modification for activity measurements throughout the various stages of purification. Substrate concentrations and assay procedure were those previously described[3] except that 4 mM cysteine was added to the incubation medium and the hydroxylamine concentration was reduced from 1 M to 0.5 M. It was also found necessary to remove heavy-metal impurities from the hydroxylamine (Merck) by treatment with 8-hydroxyquinoline[6]. Activities were estimated either from the optical change at 490 mμ

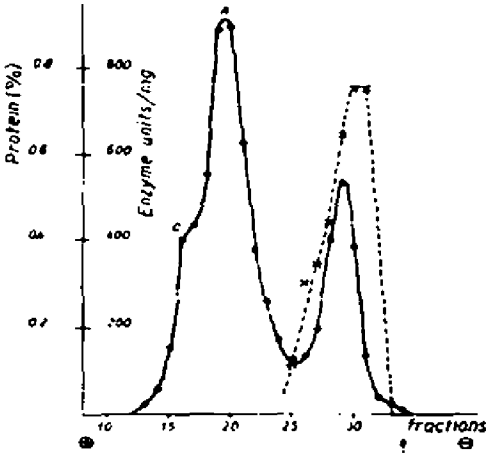

Fig. 1. Zone electrophoresis in barbitone acetate buffer (I 0.05, pH 8.2) of the 70–95% ammonium sulphate precipitate (pH 8.4) from chicken breast muscle extract. ↑, start. ●—●, protein concentration (g/100 ml). × - - - - - ×, phosphoglycerate kinase specific activity (arbitrary unit/mg protein).

TABLE I

PURIFICATION OF PHOSPHOGLYCERATE KINASE FROM CHICKEN BREAST MUSCLE

Fraction	Total protein (mg)	Purification	Recovery (%)
Muscle extract*	10 200	1	100
70–95% satn. $(NH_4)_2SO_4$ (pH 8.4)	1 000	3.8	37.6
After freeze-drying and dissolution	748	3.8	28.2
Zone electrophoresis at pH 8.2			
fraction 29	53	17.1	8.9
fraction 30–31	52	19.7	10

* From 310 g muscle.

after 25-min incubation at 37° by reference to a calibration curve established with the original extract, or from the initial velocity calculated from the progress curve of the enzymic reaction.

Under the test conditions, the most active phosphoglycerate kinase fractions eluted from the electrophoresis columns usually converted 475 μmoles substrate/ml/min/mg at 37°, thus being nearly 20 times more active than the original extract. This degree of purification appears to be close to that obtained by BUBE *et al.* (unpublished) as stated by CZOK AND BÜCHER[4] and is higher than that described by others[5]. Quantitative data for a typical enzyme preparation are given in Table I.

The purified preparations were found to be free of the following enzymic activities: α-glycerophosphate dehydrogenase (EC 1.1.1.8), acylphosphatase (EC 3.6.1.7), ATPase, diphosphoglyceromutase (EC 2.7.5.4); they contained only traces of glyceraldehydephosphate dehydrogenase (EC 1.2.1.12), and triosephosphate isomerase (EC 5.3.1.1), and showed some myokinase (EC 2.7.4.3) activity. This last contamination has been estimated to represent less than 5% of the protein content of the active fractions.

On free electrophoresis in phosphate buffer (I 0.1, pH 7.6), a preparation 18 times more active than the muscle extract (combined fractions 29 and 30, Fig. 1) showed two gradients representing 78% and 22% of the total protein. The descending mobility of the main component which contains the enzyme, was about $-0.9 \cdot 10^{-5}$ cm^2 V^{-1} sec^{-1}. The same preparation sedimented uniformly in the ultracentrifuge with a coefficient $s_{20,w} = 3.4 \cdot 10^{-13}$ sec, close to the value $3.2 \cdot 10^{-13}$ sec which has been determined for the yeast enzyme[6]. The absorbancy coefficient at 280 mμ was low, as for the yeast enzyme[2], but still varied from preparation to preparation. The enzyme was found to be activated by cysteine and even more by EDTA which also activates the rabbit-muscle enzyme[5].

Laboratory of General Biology,
University of Liège, Liège (Belgium)

C. GOSSELIN-REY

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Received July 6th, 1962