RAPID METHOD OF DETERMINING γ-AMINOBUTYRATE – α-KETOGLUTARATE TRANSAMINASE ACTIVITY

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A method of determining γ-aminobutyrate – α-ketoglutarate transaminase activity based on interaction of succinate semialdehyde, formed during the enzymic reaction, with 3-methyl-2-benzthiazolone-2-hydrazone, is described.

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This paper described a rapid method of determining activity of γ-aminobutyrate – α-ketoglutarate transaminase (GAKT), transferring an amino group from γ-aminobutyric acid (GABA) to α-ketoglutaric acid. The activity of this enzyme is determined from the increase in glutamic acid or succinate semialdehyde (SSA) formed during the reaction. However, existing methods either are complicated and require much time or they need special apparatus. For instance, when a chromatographic method is used to determine GAKT activity the results of the analysis are not known until 1 or 2 days later. The method of Baxter and co-workers [1], based on determination of glutamic acid by formation of a chelate complex with copper ions, is quicker but this method is cumbersome and unsuitable for use in batch analysis. In the literature, highly sensitive fluorometric methods described by Salvador and co-workers [4], and Pitts and co-workers [3], and a radiometric method proposed by Waksman and co-workers [6] have been described. However, despite the advantages of these methods, their use is limited to laboratories possessing the necessary fluorometric or radiometric apparatus. The method now proposed is based on the reaction described by Sawiski and co-workers [5] for determination of carbonyl compounds by means of 3-methyl-2-benzthiazolone-2-hydrazone (MBTH). Formation of a colored compound between MBTH and SSA lies at the basis of determination of the latter, and it takes place in two separate reactions: condensation of the MBTH molecule with SSA, and a process of formation of a colored derivative with the participation of ferric ions and a second molecule of MBTH. In organic chemistry experience has now been gained of the determination of several carbonyl compounds by means of this reaction [2].

TABLE 1. Reproducibility of Measurements of Activity of Partially Purified GAKT Preparation from Rat Brain (activity expressed in μmoles SSA formed during 1 h, per mg protein)

Determination No.	Activity	Determination No.	Activity
1	16,5	8	15,0
2	15,0	9	15,5
3	15,5	10	14,6
4	13,7	11	13,8
5	15,3	12	14,7
6	15,0	13	14,6
7	14,2	14	15,2

The main difficulty in the use of this reaction for determining GAKT has been the need to choose conditions under which an excess of α-ketoglutaric acid over the amount capable of reacting with MBTH would not interfere with the accuracy of SSA estimation.

SSA was obtained by hydrolysis of 2,4,6-tris-(β-cyanoethyl)-trioxan. The concentration of SSA was determined by the bisulfite method. A standard solution of SSA was kept in a frozen state at −20°. A 1% solution of MBTH was prepared before the experiment. A 0.25% solution of ferric chloride was prepared by dissolving the salt in 0.01 N hydrochloric acid.

To carry out the enzymic reaction a mixture was used containing (in 1.5 ml) the required amount of enzyme, 50 μmoles α-ketoglutaric acid, 50 μmoles GABA, 20 μg pyridoxal-5-phosphate, 20 μg reduced glutathione, and 200 μmoles tris buffer (pH 8.4). The enzymic reaction was carried out at 37°, and stopped after 1 h by addition of 0.5 ml of 20% trichloroacetic acid.

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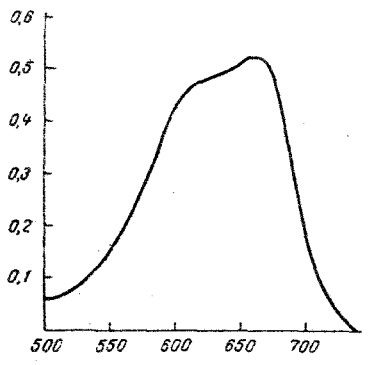

Fig. 1. Light absorption spectrum by colored derivative of SSA and MBTH. Ordinate, optical density (in extinction units); abscissa, wavelength (mμ).

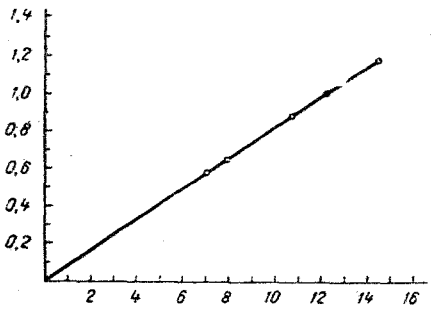

Fig. 2. Calibration graph for determining SSA concentration. Ordinate, optical density (in extinction units); abscissa, content of SSA in sample ($\times 10^{-8}$ mole).

The precipitated proteins were removed by centrifugation for 5 min at 5000 rpm. Next, 10-100 μl of deproteinized reaction mixture containing 0.02-0.16 μmole SSA was added to 0.5 ml of 1% MBTH solution. After standing for 5 min, the samples were placed on a boiling water bath, incubated for 3 min, and then cooled with water at 22-25° for 5-8 min. The cooled samples were then treated with a 1 ml 0.25% ferric chloride solution, and 5 min later 4 ml acetone was added and the mixture shaken.

Spectrophotometric measurements were carried out for 1 h after development of the color on the SF-4A spectrophotometer in a quartz cuvette 1 cm wide. It is clear from Fig. 1, which shows the spectrum of the colored derivative of SSA with MBTH, that the optical density of the samples is best determined at 660 mμ. The calibration graph (Fig. 2) was obtained under conditions identical with those in which the enzymic activity was determined, but instead of active enzyme, an inactivated preparation was used. The reproducibility of measurements of activity of a partially purified GAKT preparation from rat brain is satisfactory (Table 1), the coefficient of variance being 3.3%. The whole procedure of determining activity of the enzyme takes 1.5-2 h.

LITERATURE CITED

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