Use of Amino Acid Analyser for Determination of Aminotransferase Activities

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Authors describe the determination of some aminotransferases (alanineaminotransferase (ALT), aspartateaminotransferase (AST)) activities at 37 °C and different pH using an amino acid analyser AAA 339-T (Mikrotechna, Prague).

Metals in living system contribute to metabolism usually in form of complex species with large molecules (enzymes) or small molecules (like amino acids, and others). Low molecular weight complex species can act as substrates for some enzymes, as it was studied by [1]. In this study we have chosen aminotransferases, because their substrates are amino acids and oxo-acids, which both form complex species with metals. The determination of ALT, AST activities was performed by Bio-La-Tests (Lachema, Brno). However, the tests are not convenient for some metals (especially trace elements), because the colour development occurs in alkaline region where many metal hydroxides precipitate [2]. To remove the interferrence caused by precipitation, other methods for measurement of aminotransferase were used.

The aim of our work was to evaluate the determination of some aminotransferases by means of an amino acid analyser.

Chemicals used were p.a. Solutions were prepared in redistilled water. The program of amino acid analyser AAA 339-T for hydrolysates was shortened from 120 min to 80 min with just one sodium citrate buffer pH 3.00 [3]. Temperature for separation was 49 °C and for regeneration of ionex column with 0.3 mol/l NaOH was 61 °C. Others parameter were not changed. Taurine was used as internal standard. The enzyme reaction was stopped by addition of 20% sulfosalicylic acid solution (1 part) in the ratio 9:1 [4]. Activities of ALT and AST were calculated from increased glutamate concentration and decrease of alanine or aspartate, respectively.

The activity of aminotransferases at 37 °C and different pH were determined by Bio-La-Tests (Lachema, Brno) for comparison.

In Fig. 1 is shown typical chromatographic analysis of standard amino acid solution (1A), separation of a sample with taurine (internal standard), glutamic acid (a product) and alanine (a substrate) (1B) and separation of diluted sample with taurine, traces of glutamic acid and alanine (1C). For enzyme acivities are used usually spectrophotometric methods [e.g.

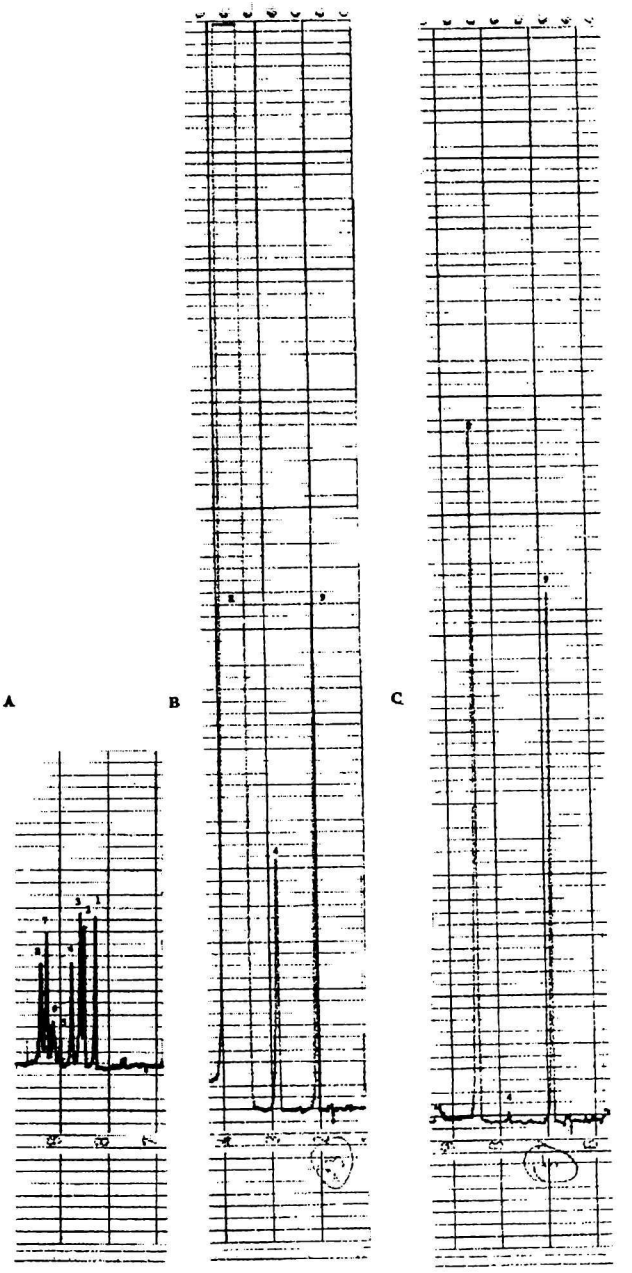

Fig. 1. Amino acid analysis of A) standard amino acid solution, B) 50 × diluted sample after 60 min incubation at 37 °C – ALT activity determination, C) non-diluted same sample. Identification of peaks: 1 – aspartic acid, 2 – threonín, 3 – serín, 4 – glutamic acid, 5 – proline, 6 – cysteine, 7 – glycine, 8 – alanine, 9 – taurine (internal standard)

1], chromatographic methods are not very common. Amino acid analyser may be used for determination of aminotransferase activities, because substrates and products are amino acids. Hence, the activity of the enzyme can be calculated from their increase (product) or decrease (substrate)

Both methods (present one and Bio-La-Test) after standardization gave for ALT and AST activities good agreement. The chromatographic method is more time consuming, but it is not sensitive enough for presentation of small quantities of trace metals in complex species with substrates.

The effect of metals in complex species with substrates on activity of ALT and AST will be published later.

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