L-Homoserine O-acetyltransferase (*Ca*Met2p) activity and inhibition.

Objectives: Identification of optimal conditions for L-homoserine O-acetyltransferase activity determination as well as selected compounds inhibitory potential evaluation.

Methods: Homoserine O-acetyltransferase (*Ca*Met2p) activity assay was conducted using 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) method. Due to optimization of activity assay we used different substrates concentrations (L-homoserine and acetyl-CoA), reaction times and purified enzyme amounts (up to 300 nM). Optimal pH for the enzyme activity was determined according to the activity assay procedure with changing pH of used buffers: Tris-HCl (pH 8.5 – 9.0), HEPES (pH 7.0 – 8.0), $NaHPO_4$ (pH 6.0 – 8.0), glycine (pH 8.5 – 11.0). The kinetic parameters were determined by measuring the activity of 300 nM protein according to the activity assay procedure. The reaction mixture contained various concentration of substrates: 0 - 4mM acetyl-CoA and 0 – 6 mM L-homoserine (L-Hom) at constant concentration of other substrate 10 mM L-Hom and 3.5 mM acetyl-CoA, respectively. For the assessment of the inhibitory potential constant concentrations of inhibitors (2 – 20mM) were added to the activity reaction buffer. The reaction was started by addition of 300 nM of enzyme. The influence of the inhibitor on the activity of the enzyme was determined by the measurement of the difference in the absorbance at 412 nm.

Results: The best results for activity determination were obtained with the use of 300 nM of isolated enzyme supplemented with 20 % (by volume) of glycerol. Standard incubation mixtures contained 3.5 mM AcCoA lithium salt and 10 mM L-Hom in 50 mM sodium phosphate buffer (pH 8.0) containing 100 mM NaCl and 1 mM EDTA in a total volume of x mL. Reaction was started by the addition of the enzyme (x µL of enzyme solution, 300 nM in 20% glycerol) and carried out for 5 minutes at 37°C. Reaction was stopped by the addition of 100 µL of Quenching buffer (100 mM sodium phosphate buffer (pH 5.8), 3.2 mM guanidinium-HCl. The amount of the CoA formed was quantified by the addition of 20 µL of 3 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) dissolved in 100 mM sodium phosphate buffer (pH 6.8) containing 10 mM EDTA. 150 µL of the mixture was transferred to a 96-well plate and measured spectrophotometrically at 412 nm at microplate reader. Of all analyzed so far compounds the best inhibitory potential was determined for L- and D-Penicillamine (L- and D-Pen).