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Kynureninase determination

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Abstract

Kynureninase determination

This enzyme methodology is available in Fungal Genetics Reports: https://newprairiepress.org/fgr/vol14/iss1/21

Vukosavovich, M., J. R. Turner and W. K. Matchett. A direct method of assaying kynureninase.

A rapid method for assaying kynureninase has been developed. As in the procedure described by Jakoby and Bonner (1953 J. Biol. Chem. 205:699), the method involves detection of anthranilic acid produced by measuring the intensity of its fluorescence. Extracts prepared from tryptophan-induced wild type strain 74A were used as a source of kynureninase. The activity was purified approximately 4-fold by ammonium sulphate precipitation and Sephadex chromatography.

The reaction mixture contained 0.16 µmole pyridoxal phosphate, 3 µmole magnesium sulfate, 0.06 µmole kynurenine, 50 µmole Tris-(hydroxymethyl) aminomethan pH 8.0, and protein (50-500 µg) in a final volume of 1.2 ml. The reaction was conducted at 30°C in the cuvette of an Aminco Bowman spectrofluorimeter equipped with thermal spacer. Anthranilic acid which appeared during the reaction was detected by measuring the intensity of its fluorescence at 409 nm. Activation was at 315 nm.

The amount of anthranilic acid produced is linear with time for about 5 minutes, after which the rote decreases. The velocity of the reaction is proportional to protein concentration from 0.045 to 0.54 mg/ml and extrapolates to zero at zero protein concentration. The velocity of the reaction approached a maximal value when the kynurenine concentration was 0.05 µmole/ml. Kynur-

enine inhibits the reaction in concentrations greater than 0. 16 μmole/ml. The rate of conversion of kynurenine to anthranilic acid was measured at kynurenine concentrations from 5 x 10^{-3} μmole/ml to 1 x 10^{-1} μmole/ml using the direct method. A standard Lineweaver-Burke plot of the data describes a line with on intercept = 0.133, slope = 1.88 x 10^{-6}, correlation coefficient = 0.99. The Km calculated from these data was 1.42 x 10^{-5}, which is essentially the same as that published by Jakoby and Bonner.

It has previously been reported that magnesium ions activate the enzyme at all stages of purification. The assays reported here have no magnesium requirement. Traces of magnesium present in the water could activate the reaction; however, EDTA had no effect on the rate. The reaction proceeds without pyridoxal phosphate; however, the amount of anthranilic acid produced is not linear with time. Successive additions of pyridoxal phosphate decrease the initial rate slightly, but the amount of anthranilic acid produced becomes a linear function of time. At 0.13 μM/ml the rate was constant for about 5 minutes. Increasing the concentration to 0.53 μM/ml decreased the rate to 1/3 of the value observed at 0.13 μM/ml.

The time required for the assay by this method is less than 10 minutes. - - - Biology Department, Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, Washington 99352.