Lysine decarboxylase assay with cucurbituril (cucurbit-7-uril)

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Method Article

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Abstract

Introduction

Amino acid decarboxylases, which have been previously assayed with laborious manometric, radiometric or multi-step colorimetric methods, can be assayed by the simple addition of a commercial macrocycle \(cucurbit-7-uril) and a fluorescent dye \(Dapoxyl) to the enzymatic mixture. The protocol refers to lysine decarboxylase, but the activity of several other amino acid decarboxylases can be similarly determined.

Reagents

Cucurbit-7-uril \(Sigma-Aldrich) Dapoxyl \(Molecular Probes, Eugene, Oregon) Lysine decarboxylase \(partially purified 1.6 U/mg, Sigma-Aldrich) 10 mM NH4OAc, adjusted to pH 6.0 with HCl and NaOH L-lysine

Equipment

Varian Eclipse spectrofluorometer \(exc = 336 nm, em = 380 nm) with temperature controller \(25 °C) 1-ml cuvette.

Procedure

1. Prepare the following stock solutions in 10 mM NH4OAc, pH 6.0: 500 µM cucurbituril, 25 µM dapoxyl, 1 mM lysine, 800 µg/ml lysine decarboxylase. 2. Add 100 µl Dapoxyl stock \(25 µM) and 780 µl buffer to the cuvette and place the cuvette into the fluorometer. Start recording in the kinetic mode \(exc = 336 nm, em = 380 nm). 3. Add 20 µl of cucurbituril stock \(500 µM) to the solution and wait ca. 2 min until the fluorescence intensity has equilibrated. 4. Add 50 µl of lysine stock \(1 mM) to the solution. 5. Add 50 µl of lysine decarboxylase \(800 µg/ml) to initiate the reaction and record fluorescence until no change in fluorescence intensity is registered. 6. Total assay volume is 1 ml. Final concentrations are: 2.5 µM Dapoxyl, 10 µM cucurbituril, 50 µM lysine, 40 µg/ml lysine decarboxylase. Steps 2-5 can be repeated with varying enzyme, substrate, or cucurbituril concentrations.

Timing

10 min for steps 2-5. The reaction should be finished under the specified conditions in < 20 min

Critical Steps

The pH of the buffer needs to be thoroughly adjusted, since the fluorescence intensity before addition of enzyme varies strongly upon slight changes in pH.

Troubleshooting

The sequential addition steps specified under “Procedure” provide an internal control. The following observations were made: After step \(2), one should observe a very low fluorescence. If the fluorescence is too high readjust the settings of the fluorometer. After step \(3), the fluorescence intensity should immediately increase ca. 10-20-fold. If the fluorescence increase is very different, check the pH of the buffer. After step \(4), the fluorescence should not change by more than 10%. If it changes by a larger amount, check the lysine sample for impurities. After step \(5), the fluorescence should decrease first stepwise \(immediately) and then more slowly with time. If the latter time-resolved fluorescence decay is not observed after 5 min, the enzyme may be inactive. If the stepwise \(immediate) decrease in fluorescence intensity in step \(5) is very pronounced \(we relate this to varying degrees of salts and impurities in the enzyme sample), the sequence of steps \(4) and \(5) can be exchanged to obtain visually improved enzyme kinetic traces. In this case the time-resolved fluorescence decay is only observed after the addition of substrate in step \(5), while upon addition of enzyme in step \(4) only an immediate stepwise decrease in fluorescence intensity is observed.

Anticipated Results

The fluorescence should decrease in a time-dependent manner \(in ca. 10-20 minutes by a factor of ca. 3)

References

A. Hennig, H. Bakirci, W. M. Nau, Label-Free Continuous Enzyme Assays with Macrocycle-Fluorescent Dye Complexes, _Nature Methods_ \(2007) in press