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Biochemistry

Induction of Intracellular Glucose Oxidase outside the Cell

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ABSTRACT. The range of Intracellular Enzymes is rather high, and often organisms synthesize enzymes inside of a cell without developing them outside it, into the liquid culture. In this work, it is shown by us that by adding various salts with Mulberry root extract into the liquid culture, it is possible to achieve the Intracellular Enzyme appearing outside of a cell in the liquid culture. Of course, various organisms will react to various salts in different ways, but in general, such an approach gives positive results. Asperilius Niger – an organism producing Intracellular Glucose Oxidase - was taken as an example. *© 2016 Bull. Georg. Natl. Acad. Sci.*

Key words: intracellular enzymes, enzyme biosynthesys, glucose oxidase.

Very often, both in production and in the scientific sphere, there is a need for obtaining enzyme which is synthesized by microorganisms only inside of a cell and is not produced into the liquid culture. Earlier it was shown [1,2] that depending on the temperature, organisms can synthesize various enzymes vastly different from each other in terms of thermal stability and having various molecular weights and isoelectric points. For induction of the enzyme outside of a cell, different salts were used by us (Table 1), which were added to a culture medium in different concentrations. The activity of Glucose Oxidase in the liquid culture was measured (Table 1).

Results and Discussion

For induction of the enzyme to the outside the cell, we have used liquid media with Mulberry root extract and various salts (Table 1), which were added to the culture medium with various concentrations. Activity of glucose oxidase [3-6] was measured in liquid culture.

As can be seen from (Table 1), certain salts like $Ca(NO_3)_2$ induce the discharge of glucose oxidase from the cell into the liquid culture. It is also worth noting that different salts affect the induction of glucose oxidase differently, but $Ca(NO_3)_2$ being the most effective among them.

Table 1. List of salts for induction of glucose oxidase

Salt	Concentration	Activity μ/mg protein
Potassium sodium tartrate tetrahydrate $KNaC_4H_4O_6 \cdot 4H_2O$	0.15%	-
Potassium permanganate $KMnO_4$	0.15%	-
Potassium ferrocyanide $K_4[Fe(CN)_6]$	0.15%	1.5
Potassium ferricyanide $K_3[Fe(CN)_6]$	0.15%	2.0
Calcium nitrate $Ca(NO_3)_2*4H_20$	0.15%	8.7

Table 2. Induction effect dipending on salt concentration

Salt	Concentration	Activity μ/mg protein.	Concentration	Activity μ/mg protein.	Concentration	Activity μ/mg protein.	Concentration	Activity μ/mg protein.	Concentration	Activity μ/mg protein.
$Ca(NO_3)_2*4H_20$	0.075%	4	0.15%	8.7	0.3%	17	0.6%	15	1.2%	9

Later it was shown, that by increasing $Ca(NO_3)_2$ salt, the induction effect of plant extract increases up until the concentration of 0.3 mg/ml, and by increasing the concentration of $Ca(NO_3)_2$ above 0.3 mg/ml, the induction effect starts to drop rapidly (Table 2).

Materials and Methods

For growing Asperilius Niger we use liquid medium with the following concentrations: glucose - 10%, citric acid - 0.075%, $Ca(NO3)_2$ *4H20- 0.3 % (0.2%), KH2PO4 - 0.025%, MgSO4*7H2O - 0.025%, KCl- 0.025%, FCl3*6H2O- 0.001%, NaNO3- 0.3%. 0.5% Mulberry root extract as an inductor.

In order to determine the activity, we use the following procedure:

1. Pipet 3.0 ml of reaction mixture (Reagent E) into a 1.0 cm^2 cuvette.

2. Place the cuvette into the spectrophotometer and allow the temperature of the reaction mixture to equilibrate to 25 ± 0.5°C. Subsequently, record the blank rate for 2 minutes.

3. Add 0.025 ml of the enzyme (Solution F). Mix. Record the absorbance change for 5 minutes.

4. Determine the absorbance change (DA) per minute over a 3 minute period. Subtract the blank rate if necessary.

Calculations.

$$U/ml = \frac{(DA'/min)(Vt)(dilution)}{(\varepsilon_{510})(Vs)}$$

Where:

DA' = Corrected absorbance change

Vt = Total assay volume, 3.025 ml

ε_{510} = Millimolar extinction coefficient for quinoneimine dye, 6.584

Vs = Sample volume, 0.025 ml

Specific Activity.

Calculate the specific activity as follows:

$$U/mg = \frac{U/ml}{mg/ml\ protein[per\ Biuret]}$$

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უჯრედშიდა გლუკოზა ოქსიდაზის ინდუცირება უჯრედს გარეთ

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უჯრედშიდა ფერმენტების სპექტრი საკმაოდ მაღალია და ხშირად ორგანიზმები ასინთეზირებენ ფერმენტებს უჯრედის შიგნით, რის შედეგადაც ისინი არ გამოიმუშავებენ მას უჯრედის გარეთ, კულტურალურ სითხეში. ამ სამუშაოში ჩვენ მიერ ნაჩვენებია, რომ სხვადასხვა სახის მარილების დამატებით თუთის ფესვების ექსტრაქტთან ერთად კულტურალურ სითხეში, უჯრედშიდა ფერმენტი შესაძლოა აღმოჩნდეს უჯრედის გარეთ, კულტურალურ სითხეში. რასაკვირველია სხვადასხვა სახის ორგანიზმები განსხვავებულად რეაგირებენ სხვადასხვა მარილებზე, მაგრამ საბოლოო ჯამში ამ სახის მიდგომა გვაძლევს დადებით პასუხს. ნიმუშის სახით აღებულ იქნა ორგანიზმი Asperilius Niger, რომელიც წარმოქმნის უჯრედშიდა გლუკოზა ოქსიდაზას.

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