

EXP. NUMBER 12	EXPERIMENT/SUBJECT Peroxidase immobilization	DATE 7/22/09	27
NAME Jason Largent	LAB PARTNER Tim Van	LOCKER/DESK NO.	COURSE & SECTION NO. Biochem Lab

Title Activity & Thermal Stability of Gel-Immobilized Peroxidase.

Reference: Experiment 12 in Modern Exp. Bio Chem 3rd Slides on blackboard.

Synopsis: Enzymes provide great benefits to bio chem researchers as they are highly effective catalysts. In commercial use, however, they are very expensive & difficult to use & control some times, we can immobilize the enzyme to simulate *in vitro* conditions and better control the reaction, as well as re use the enzyme. Here we entrapped the enzyme in a cross linked polymer of acrylamide gel & methylene bisacrylamide, ^{non-radical} peroxidase will be used to catalyze the breakdown of peroxide into water using aminoantipyrine-phenol as an electron donor. Various levels of trapped enzyme were compared to free enzyme assays. Both enzyme forms are then heated to 70°C & the reaction rates are again compared to see the thermal stability.

Procedure

immobilizing the enzyme
 in order, mix in a 50ml screw cap tube:
 3.25ml potassium phosphate buffer
 2.7ml acrylamide/bis acrylamide solution
 1.0ml of 0.1mg/ml peroxidase
 - invert mix
 100ml 10% ammonium persulfate
 - vortex mix
 50ml TEMED
 invert & vortex

Observations

Only required 50ml TEMED
 took ~ 2min to react, then instant gel

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EXP. NUMBER	EXPERIMENT/SUBJECT	DATE	28
NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.

Procedure

the gel is washed,
vacuum filter if needed

Place gel in beaker, add
10 mL water, aspirate
w/ pipet 8-10 times,

filter on Buchner funnel
w/ vacuum, allow to filter
5 min to dry,
weigh gel

Observations

No need to do first vacuum
filter.

tarred beaker 30.1786g
beaker + gel 40.0801g
gel 9.9015g

enzyme assay

Six tubes, set up

#1, #2 2.50 mL phenol reagent
+ 0.05g gel

#3 + 4 2.50 mL phenol + 0.10g gel

#5 + 6 2.50 mL phenol + 0.20g gel

odd # tubes are allowed to
react for < 10 sec

even # react for 3 min w/
constant invert mix,

add 2.50 mL of H₂O₂ to each
tube @ prior to measure. @

510 nm

after reacting, push thru

filter to remove enzyme

& stop rxn,

then measure.

	g gel	A ₅₁₀	time reacted
#1	0.0573	-0.020	0:00 min
#2	0.0500	0.297	3:00 min
#3	0.1048	0.003	0:00 min
#4	0.1042	0.654	3:00 min
#5	0.1987	0.008	0:00 min
#6	0.1974	0.864	3:00 min

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EXP. NUMBER	EXPERIMENT/SUBJECT	DATE
NAME	LAB PARTNER	COURSE & SECTION NO.
	LOCKER/DESK NO.	

Procedure

Observations

Free enzyme

Blank = 2.5 mL Phenol + 2.5 mL DI

3 tubes, no filtering
dilute enzyme, 100 μL to 200 μL buffer

- #1 2.50 mL Phenol react + 10 μL free enzyme
- #2 2.50 mL Phenol + 20 μL enzyme
- #3 2.50 mL Phenol + 40 μL enzyme

	A540
#1	0.500
#2	0.854
#3	0.1708 1.708

add 2.50 H₂O₂ + let react for 3 min, then measure A510

Thermal Stability

dilute 10 μL enzyme w 2990 μL Buffer.

2 tubes, 1 mL of ↑ in each, heat in 70° bath 4 min, then cool, 1 tube

add 2.0 mL Phenol + 2.0 mL H₂O₂ to each tube, let react for 3 min, A510

Free	A510
Cool =	0.598
hot =	0.657

immobilized,
weigh 0.1g gel into 2 tubes,
w/ 0.5 mL buffer
heat one tube to 70°C for 4 min,
cool,
add 2.25 mL Phenol + 2.25 mL H₂O₂
mix for 3 min, then filter,
A510

immobilized	weight (g)	A510
cool =	0.1010	0.561
heated	0.0977	0.491

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Calculations

activity of free enzyme + immobilized enzyme
 $\frac{\Delta A/min}{6.58 \times 0.01 \times 1000}$

free = $\frac{\Delta A/min}{6.58 \times 0.010 \times 1000}$

#1 $\frac{0.09967}{(6.58)(0.05365)} = 0.2823$

#1 $\frac{0.1667}{(6.58)(.01)(1000)} = 0.2533$

#2 $\frac{0.217}{(6.58)(0.1045)} = 0.3156$

#2 $\frac{0.2847}{(6.58)(.01)(20)} = 0.2163$

#3 $\frac{0.2853}{(6.58)(0.19805)} = 0.2189$

#3 $\frac{0.5693}{(6.58)(.01)(40)} = 0.2163$

Avg = $\frac{0.2823 + 0.3156 + 0.2189}{3} = 0.2723$

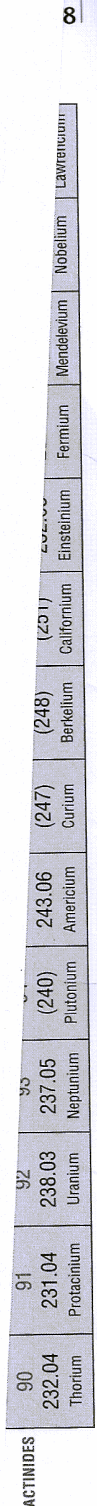
Avg = $\frac{0.2533 + 0.2163 + 0.2163}{3} = 0.2286$

% activity Remaining

immobilized
 $\frac{AA@70^\circ}{AA@RT} \times 100\%$

$\frac{.491}{.561} \times 100 = 87.52\%$

$\frac{0.657}{0.598} = 109.9\%$



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