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12	Peroxidase immobilization	7/22/09

NAME	LAB PARTNER	LOCKER/DESK NO.	COURSE & SECTION NO.
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Title: Activity & Thermal Stability of Gel-Immobilized Peroxidase.

Reference: Experiment 12 in Modern Exp. Biochem 3rd
5 slides on blackboard.

Synopsis: Enzymes provide great benefits to biochem 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, horseradish peroxidase will be used to catalyze the breakdown of peroxide into water using amino antipyrine-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
2.5mL acrylamide/bis acrylamide solution
1.0mL of 0.1mg/mL peroxidase
— invert mix
100µL 10% ammonium persulfate
— vortex mix
50µL TEMED
invert & vortex

Observations

Only required 50µL TEMED

took ~2min to react, then instant gel

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Procedure

the gel is washed, Vacuum filter if needed

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

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

weigh gel

Observations

No need to do first vaccum filter.

tared beaker 30,1786g
beaker + gel 40,0801g
gel 9,9015g

enzyme assay

Six tubes, set up

#1, #2 2,50mL 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 2,50mL of H_2O_2 to each tube @ prior to measure @ 510nm
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

Procedure

Observations

free enzyme

Blank = 2.5 mL Phenol + 2.5 mL DI

3 tubes, no filtering
dilute enzyme, 100 µL to 900 µL buffer

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

add 2.50 H_2O_2 + let react for 3 min, then measure A_{510}

	A_{540}
#1	0.500
#2	0.854
#3	~~0.1708~~ 1.708

thermal Stability

dilute 10 µL enzyme w 2990 µL Buffer.

2 tubes, 1 mL of ↑ in each,
heat 1 in 70° bath 4 min, then cool
1 tube
add 2.0 mL Phenol + 2.0 mL H_2O_2 to each tube, let react for 3 min, A_{510}

	Free A_{510}
Cool =	0.598
hot =	0.657

immobilized,
weigh 0.1 g 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 H_2O_2
mix for 3 min, then filter,
A_{510}

Immobilized	Weight (g)	A_{510}
Cool =	0.1010	0.561
heated	0.0977	0.491

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Calculations

activity of free enzyme & immobilized enzyme

immobilized $\frac{\Delta A/min}{6.58 \times 8 mg\ gel}$

free = $\frac{\Delta A/min}{6.58 \times 0.010 \times \mu L\ enzyme}$

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

#1 $\frac{0.1667}{(6.58)(.01)(\cancel{10})} = 0.2533$

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

#2 $\frac{\cancel{0.248}\ 0.2847}{(6.58)(.01)(20)} = 0.2163$

#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

$\frac{\Delta A @ 70^\circ}{\Delta A @ RT} \times 100\%$

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

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

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