

Purification of P-P exchange Enzyme

13 May

15 ml YE + 30 ml cold H₂O. whole string added 28.5 ml EtOH keeping temp between 7-10°. Spun ppt down for 4 min at 100 and extracted ppt with 20 ml Tris 4/10 pH 8.0. The insoluble material was centrifuged & discarded. To the sup was added 10 ml 0.5M succ pH 6.1 and then cooled to -1° and 8.2 ml EtOH (-15°) was added dropwise keeping temp 0-1°. The ppt was centrifuged and dissolved in 15 ml Tris. (EtOH₂) To this solution was added 6 ml Co gel and after 5 min the gel was centrifuged down. The gel was washed first with 15 ml of .075M PO₄ pH 7.5 and then with 10 ml. The two gel eluates were combined and (.25 ml) and 8.2 gm AS were added. After 3 min the ppt was centrifuged down and dissolved in 3 ml Tris. To the sup added 3.1 gm AS and after 3 min the ppt spun down and dissolved in 3 ml Tris

EtOH₂ = 15 ml
 1st AS = 3.0
 2nd AS = 3.1
 sup = 30

Assay

| | cpm/mt. | Total | ³² P 1.34 | units/mt. | Q | Total units |
|---------------------------------|---------|-------|----------------------|-----------|-----|-------------|
| 1) Dil YE 1-25 .05 ml | 990 | 2970 | 4.7 | .25 | 211 | |
| 2) EtOH ₂ 1-25 .05 | 1520 | 4560 | 7.2 | 2.3 | 108 | |
| 3) 1 st AS 1-215 .05 | 244 | 747 | 3.5 | 1.3 | 11 | |
| 4) 2 nd AS 1-225 .05 | 910 | 2730 | 21.6 | 9.2 | 67 | |
| 5) AS sup .03 | 370 | 1110 | .58 | | 17 | |
| | | | | | 95 | |

Proteins

| | D 660 | γ Protein/mt. |
|---------------------------|-------|---------------|
| 1) Dil YE 1-25 .02 | 435 | 18.8 |
| 2) 1 st AS .02 | 318 | 2.7 |
| 3) 2 nd AS .02 | 275 | 2.35 |
| 4) EtOH ₂ .02 | 358 | 3.1 |

Treatment of Boiled Juice

BJ prepared 30 April (1 part yeast + 3 parts 0.1M KHCO_3 autolyzed 4 hrs, spun and the extract heated in boiling H_2O for 2 min, cooled and ppt spun off).

I

1 ml + 1 ml conc HCl (6M) in tube. sealed (shorter tube)
Contents Rump 2x, filtered, neutralized and diluted to 2 ml. (deep red solution)

II

1 ml + 1 ml 2M HCl

Removed 1 ml and neutralized to BTB.

The remaining portion was heated in a boiling H_2O bath for 10 min and the neutralized to BTB

III

10mg of crystalline bovine serum albumin dissolved in 1 ml. 1 ml conc HCl added and placed in sealed tube (larger tube). Hydrolysate evaporated on steam bath to dryness. Dissolved in 1 ml H_2O , neutralized and diluted to 1 ml.

Study of Boiled Juice Effect.

May 14

Concentration of BJ. giving maximum effect

System

0.8 ml Tris

0.05 ml hot P-P (.09M)

0.02 ml 0.1M ATP

0.05 ml 0.2M $MnCl_2$

0.05 ml AS 1-12

| | cpm/ml - Bkd | Total |
|-------------------------|--------------|-------|
| 1) - | 31 | 93 |
| 2) 0.01 ml BJ | 410 | 1230 |
| 3) 0.02 ml BJ | 8770 | 2310 |
| 4) 0.04 ml BJ | 1360 | 4080 |
| 5) 0.06 ml BJ | 1220 | 3660 |
| 6) 0.1 ml BJ | | |
| 6) 0.05 ml Dialyzed BJ | 25 | 75 |

Bkd 35

From this experiment seems that this amount of enzyme is saturated at about .04 ml BJ and the dialyzed BJ (dialyzed overnight vs. cold tho) is completely inactive.

AS
.05 ml E₁₂ 1-12II

| | cpm/ml. | Total |
|-----------------------------------|---------|-------|
| 1) .08 ml BJ heated 10 min 1M HCl | 750 | 2250 |
| 2) .08 ml BJ " " " + .04 ml BJ | 980 | 2940 |
| 3) .04 ml Bov. Alb Hyd | 275 | 825 |
| 4) .04 ml " " + .04 ml BJ | 1320 | 3960 |
| 5) .08 ml Hydrol. BJ. | 981 | 2943 |
| 6) .08 ml " " + .04 ml BJ | 970 | 2910 |