Purification of P-P exchange Enzyme

13 May

15 ml YE + 30 ml cold H_2O. While stirring 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 M/20 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 8.8 ml EtOH (-15°) was added dropwise keeping temp 0 - -1°. The ppt was centrifuged and dissolved in 15 ml Tris. ($EtOH_2$) To this solution was added 6 ml Ca gel and after 5 min the gel was centrifuged down. The gel was washed first with 15 ml of .075 M PO_4 pH 7.5 and then with 10 ml. The two gel eluates were combined (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

...ms.
$EtOH_2$ = 15 ml.
1st AS = 3.0
2nd AS = 3.1
sup = 30

Assay

P^{32} 1.34

		cpm/ml.	Total	units/ml.	Q	Total units	
1) Dil YE 1→5	.05 ml	990	2970	4.7	.25	211	
2) $EtOH_2$ 1→5	.05	1520	4560	7.2	2.3	108	
3) 1st AS 1→15	.05	249	747	3.5	1.3	11	95
4) 2nd AS 1→25	.05	910	2730	21.6	9.2	67	
5) AS sup	.03	370	1110	.58		17	

Proteins

		D 660	γ Protein/ml.
1) Dil YE 1→5	.02	435	18.8
2) 1st AS	.02	318	2.7
3) 2nd 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 (~6 N) in tube. sealed (shorter tube)
Contents evap 2x, filtered, neutralized and diluted to 2 ml. (deep red solution)

II 1 ml + 1 ml 2.N HCl
Removed 1 ml and neutralized to BTB.
The remaining portion was heated in a boiling H_2O bath for 10 min and then neutralized to BTB

III 10 mg 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 (.04M)
0.02 ml 0.1 M ATP
0.05 ml 0.2 M $MnCl_2$
0.05 ml AS 1->12

I

		cpm/ml - Bkd	Total
1)	–	31	93
2)	0.01 ml BJ	410	1230
3)	0.02 ml BJ	770	2310
4)	0.04 ml BJ	1360	4080
5)	0.06 ml BJ	1220	3660
~~6)~~	~~0.10 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 B.J and the dialyzed BJ (dialyzed overnight vs. cold H_2O) is completely inactive.

.05 ml AS Enz 1->12

II

		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. B.J.	981	2943
6)	.08 ml " " + .04 ml BJ	970	2910