Amount of different components used in different media (mg/l)

Components	SM	M II	M III	Components	SM	M II	M III
NaCl	2100	6800	6800	KCl	1600	1100	1100
$NaHCO_3$	400	--	--	$CaCl_2$	600	400	400
$NaH_2PO_4,2H_2O$	--	150	150	$MgSO_4,6H_2O$	700	1390	1390
CH_3COONa	--	84	84	$MgCl_2,6H_2O$	--	1390	1390
$Fe(NO_3)_2,9H_2O$	--	0.72	0.72	Tryptophan	100	20	100
KH_2PO_4	450	--	--	Tyrosine	500	40	200
Na_2HPO_4	700	--	--	Valine	300	50	100
Deoxyribose	--	0.5	0.5	Malate	100	--	--
Ribose	--	0.5	0.5	α-ketoglut.	200	--	--
Sucrose	--	16000	--	Succinate	100	--	--
Glucose	2000	1000	3000	Fumarate	100	--	--
Trehalose	2000	--	2000	Vit.A acetate	--	0.1	--
Alanine	500	70	100	Menaphthene	--	0.01	--
Arginine	400	70	100	Ascorb.acid	--	0.05	--
Asp. acid	400	95	100	Riboflavine	--	0.01	--
Cystine	100	20	--	Ca pantoth.	--	0.01	--
Cysteine	60	0.1	60	Pyridox. HCl	--	0.025	--
Glut. acid	800	150	150	P.A.B.A.	--	0.05	--
Glutamine	1800	100	500	Folic acid	--	0.01	--
Glycine	250	50	100	Nicot. acid	--	0.025	--
Histidine	400	245	250	Inositol	--	0.05	--
H. proline	--	10	10	Biotin	--	0.01	--
Isoleucine	150	40	100	Choline Cl	--	0.05	--
Leucine	150	120	120	Aneurine HCl	--	0.01	--
Lysine	1650	70	500	Calciferol	--	0.1	--
Methionine	800	30	300	α-tocoph. P	--	0.01	--
β-alanine	--	50	--	Adenine HCl	--	5.1	0.3
Proline	1700	40	500	Guanine HCl	--	0.3	0.3
Serine	250	135	150	Xanthine	--	0.3	--
Threonine	350	60	100	Hypoxanthine	--	0.3	--
Thymine	--	0.3	0.3	Br. thymol blue	10	--	10
Uracil	--	0.3	0.3	Glutathione	--	0.05	--
Cytosine	--	--	0.3	FBS	10-15%	--	0.5-1%
Adenylic acid	--	0.2	--	Yeast hydrol.	2000	--	10
ATP	--	5	7	Nipagin	5	5	--
Cholesterol	--	2	--	Streptomycin	100	100	100
Tween	--	5	--	Penicillin G	200,000 units	200,000 units	200,000 units
Phenol red	--	10	--				

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Milanović, M. and W.W. Doane. Arizona State University, Tempe, Arizona. Affinity chromatographic purification of amylase from Drosophila species.

We are using an improved method for the purification of α-amylase from crude extracts of D. melanogaster and D. hydei. This simple, rapid and highly efficient method employs the affinity chromatographic technique of Vretblad (1974) as modified by Silvanovich and Hill (1976). Amylase is retained on a column of cyclohepta-amylose (CHA) epoxy Sepharose 6B. Contaminating proteins are washed through the column in an elution buffer and the amylase is selectively removed from the column by means of a CHA-containing elution buffer.

When crude extracts of larvae or flies are passed through the CHA affinity column, the amylase subsequently eluted from the column appears pure on the basis of disc electrophoresis and staining with Coomassie blue and/or starch-iodine. The CHA column thus represents a con-

siderable improvement over the previously used glycogen precipitation method of Loyter and Schram as modified for Drosophila by Doane et al. (1975). Application of the latter method results in the co-precipitation of several contaminating proteins which require an additional electrophoretic step for removal. Another disadvantage of the glycogen precipitation method, which is avoided by use of the CHA column, is the difficulty encountered in removing all of the dextrins bound to the purified amylase by autodigestion.

Steps for the purification of Drosophila amylases by CHA affinity chromatography follow. Further details of the method and its application are being prepared for publication (Milanović and Doane).

I. Preparation of Crude Amylase Extracts

1. Homogenize larvae or flies in the same buffer used for washing the affinity column (step II,5,d) at a weight:volume ratio of about 1:3. Add PTU to saturation.
2. Homogenates should be frozen/thawed and autodigested at 25°C for 2-3 hours to release additional amylase from cell debris and native glycogen. Store homogenates at -20°C or chill on ice and proceed.
3. Centrifuge homogenates at 17,000 rpm (34,800 g on Sorvall RC5 refrigerated centrifuge) for 20 minutes. Decant or pipette supernatant through glass wool to remove lipids and repeat centrifugation until the crude extract is clear.

II. Coupling of Cyclohepta-amylose (CHA) to Sepharose

1. Place 4.0 g of epoxy-activated Sepharose 6B (Pharmacia; Sigma) in a beaker and add about 25 ml of redistilled, demineralized H_2O. Stir.
2. Transfer gel slurry to sintered glass filter funnel and wash with redistilled H_2O (400 ml) for 1 hour by applying suction.
3. Wash gel with 25 ml of 0.1 M NaOH solution to remove excess liquid.
4. Transfer gel to a solution of 300 mg of CHA (Sigma) in 12 ml of 0.1 M NaOH; shake for about 19 hours in a water bath at 45°C.
5. Following incubation, wash gel on a glass filter with:
   a. 100 ml of redistilled water, 30 minutes;
   b. 200 ml of glucose solution (25 mg/ml), 30 minutes;
   c. 100 ml of redistilled water, 30 minutes;
   d. 400 ml of 0.05 M phosphate buffer, 2 hours. The phosphate buffer, pH 6.9, contains: KH_2PO_4 (3.03 g/l), Na_2HPO_4 (3.96 g/l), NaCl (0.41 g/l), and $CaCl_2$ (0.111 g/l).

III. Purification of Amylase

1. Set up column (e.g. Pharmacia column K 16/20). It should be very clean.
2. Rinse column with 0.05 M phosphate buffer.
3. Add CHA-Sepharose slurry and allow it to settle.
4. Wash column several times with starting buffer.
5. Run crude amylase extract through column and wash column immediately with 100 ml of phosphate buffer, adjusting flow rate to 25 ml/hr. (2 ml samples work well.)
6. Elute amylase off column with CHA-buffer (8 mg of CHA/ml phosphate buffer), 100 ml.
7. Collect 5 ml fractions (approximately 50 fractions), monitoring for protein at A_{280} on spectrophotometer. This takes about 8 hours.
8. Pool fractions containing amylase (typically fractions #20 or #30 to #45±).
9. Dialyze in redistilled H_2O 16-20 hours at room temperature.
10. Concentrate and/or lyophilize.

The time required for Steps III, 9 and 10 can be reduced to a few hours by use of Sartorius Membranfilter collodion bags (SM 132 00) to remove or replace the elution buffer and concentrate the purified amylase. For prolonged storage of the pure enzyme at -20°C, 0.05 M Tris-HCl buffer, pH 7.4, containing 0.003 M $CaCl_2$ proved best for retaining enzyme activity (L.G. Treat, unpubl.). Lyophilization results in some loss of activity.

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