Doane, W.W., M.M. Kolar and P.M. Smith.
Yale University, New Haven, Connecticut.

Purified α-amylase from D. hydei.

Anylase was extracted from adults of the Zurich strain of D. hydei, which is homozygous for the electrophoretic variant Amy^7. Flies were mass reared (Doane, DIS 45:189, 1970) and aged one week on a starch-yeast diet to increase enzyme production.

Partial purification was achieved through modification of the method Loyter and Schramm (Biochim. Biophys. Acta 65:200, 1962). By this method α-amylases with two or more binding sites per molecule are specifically precipitated from crude extracts as a glycogen-enzyme complex insoluble in 40% ethanol. However, some unidentified contaminating proteins were also precipitated by this means from the Drosophila material. These were eliminated by concentrating the proteins in the partially purified extract through lyophilization and then subjecting it to slab acrylamide gel electrophoresis. The apparatus of Roberts and Jones (Analyt. Biochem. 49:592, 1972) was used in a preparative manner, with 5% acrylamide, 0.1 M Tris-borate buffer, pH 9.4, and a constant voltage of 450 V for 1 1/2 hours at 7°C. Amylase migrated as a single band to a position relatively distant from contaminants so that it could be easily cut out and eluted. The eluted enzyme was dialyzed against deminized water for 16 hrs and lyophilized to dryness. Samples of purified amylase were tested for protein impurities by running them through the disc electrophoresis procedure and staining the gels with Coomassie Blue, a very sensitive protein stain. No contaminants were detected by this means.

Data on the partially purified extract from a sample of 71.3 g of flies (Doane, Kolar and Smith, Genetics 74:s64, 1973, and in prep.) are given in the table below. Two different assays were used to determine specific activities (Doane, J. Exp. Zool. 171:321, 1969); the amylase yield and purification factor for each are in fair agreement. Lowry assays for total protein indicated that only 0.3% of the soluble protein in the crude extract was present in the partially purified S_7 extract. Further analysis showed that approximately one-third of this protein was α-amylase. Hence, this enzyme makes up about 0.1% of the total soluble protein in the original crude extract, S_1.

Partial purification of amylase
Source: D. hydei, 71.3 g of flies

Extract	Protein[1]		Reduction Assay[2]			Starch-Iodine Assay[3]		
	Total	Yield	Spec. Activ.	Enzyme Yield	Purif. Factor	Spec. Activ.	Enzyme Yield	Purif. Factor
	mg	%	MU/μg	%		SU/μg	%	
Crude (S_1)	4,118.00	100.0	4.28	100.0		2.16	100.0	
Partially purified (S_7)	12.18	0.3	975.52	67.5	227.9	453.45	62.2	209.9

[1] Method of Lowry et al. (J. Biol. Chem. 193:265, 1951).
[2] 3,5-Dinitrosalicylic acid reduction assay: 1 MU = 10^{-4} μmoles maltose equiv./min. at 25°C.
[3] Starch-Iodine assay: 1 SU = 10^{-4} mg starch hydrolyzed/min. at 25°C.

The Molecular Weight of purified "α-amylase-7" was determined in SDS gels by the method of Weber and Osborn (J. Biol. Chem. 244:4406, 1969; also, Dunker and Rueckert, Ibid.: 5074). The average of four independent estimates was 54,500 daltons, with a deviation from this in any given run of < 5% (Doane and Kolar, Isoz. Bull. 7:in press). Reduction of the amylase in 1% solutions of SDS containing 1% 2-Mercaptoethanol, ± 4 M urea, gave no indication of dissociable subunits. Neither did treatment with 0.0015 M Dithiothreitol, a more powerful reducing agent than 2-Me. It is concluded that the amylase molecule in Drosophila is a monomer, as suggested by genetic analyses. (Supported by NSF grant GB 29276 and USPHS grant GM 18-72901A1).