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OBSERVATIONS ON HONEY DIASTASE

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SUMMARY

Honey diastase was separated into two major components, the first containing mainly α -amylase, the second a mixture of α - and β -amylases. These amylases probably differ from amylases of other sources.

INTRODUCTION

An examination of the literature on honey diastase reveals considerable difference of opinion as to the origin of this enzyme. Some workers have maintained that it is produced by the bee (Ammon, 1949; Braunsdorf, 1932; Neprašová & Svoboda, 1956; Weishaar, 1933); others state that it is derived from pollen or other plant materials (Fiehe, 1932; Lothrop & Paine, 1931; Vansell & Freeborn, 1929). Still others feel that it is a product of both plant and animal origins (Gorbach, 1942; Gothe, 1914). There has been no attempt to isolate and characterize this enzyme, but there is evidence for the presence of two amylases in what we refer to as honey diastase (Lampitt *et al.*, 1930; Schmidt-Nielsen & Artun, 1938).

In the course of our own studies on honey enzymes, we have attempted to isolate and purify diastase, separate it into its component amylases and characterize them. In this we have not been completely successful, but some interesting observations have been made.

All experiments to be mentioned here were performed with two unheated honeys described as fall-flower blends (HS-34 and 37), obtained from the Finger Lakes Honey Producers Cooperative*, Groton, New York, and stored at 4°C. DEAE-cellulose (N,N-diethylaminoethyl-cellulose) was obtained from Eastman Organic Chemicals, Rochester, New York, and Sephadex G-100 from Pharmacia Fine Chemicals Inc., New Market, New Jersey. α -Amylase activity was determined by the method of Schwimmer (1945), and diastase (total amylase activity) by the method of Schade as modified by White (1959). The difference between the two was attributed to β -amylase, which could not be determined directly. Protein was assayed by the method of Warburg & Christian (1942) as modified by Layne (1957). Ion-exchange chromatography was conducted according to Sober *et al.* (1956), paper chromatography according to Albon and Gross (1952).

Assuming that honey diastase could be a mixture of both α - and β -amylases (α from the bee and β from pollen or nectar), we used a crude enzyme concentrate (prepared according to White *et al.*, 1963), trying first to destroy the β -amylase

* Mention of company or trade names does not imply endorsement by the Department over others not mentioned.

by heating (Schwimmer & Balls, 1949), and then to recover the remaining α -amylase. This was without success in several attempts. Using the technique of Nadkarni and Sohnie (1963), we then tried several times to destroy the α -amylase and recover the β . This also failed. With neither treatment did any enzymatic activity of either type remain, indicating probable differences between the honey amylases and those from other sources.

Assuming that an α -amylase predominated in the mixture, a preparation designed for this type of enzyme was next attempted. It was based on the work of Markovitz *et al.* (1956) and Thayer (1953). This also ended in failure.

Turning to column fractionation, Sephadex G-100 afforded no separation, but DEAE-cellulose showed promise. Since preliminary experiments had indicated the desirability of a more refined starting material for column separation, the following procedure was devised: 200 g. of honey HS-34 were dissolved with 96 ml. water containing 48 mg. merthiolate, incubated for 30 min. at 37°C. (sufficient for sterilization; see White *et al.*, 1963), then dialysed against running tap-water for 24 hours. The dialysed honey solution (400 ml.) was cooled to 4°C. and treated with an equal volume of cold acetone to effect precipitation. The precipitate was removed by centrifugation for 10 min. at 1600 r.p.m. (4°C.), resuspended in 0.2-M sodium phosphate buffer, pH 6.1, and dialysed as above. The dialysed solution (33 ml.) was centrifuged (1600 r.p.m. for 10 min.) to remove a small precipitate, and treated with enough ammonium sulphate to give 0.2 saturation at

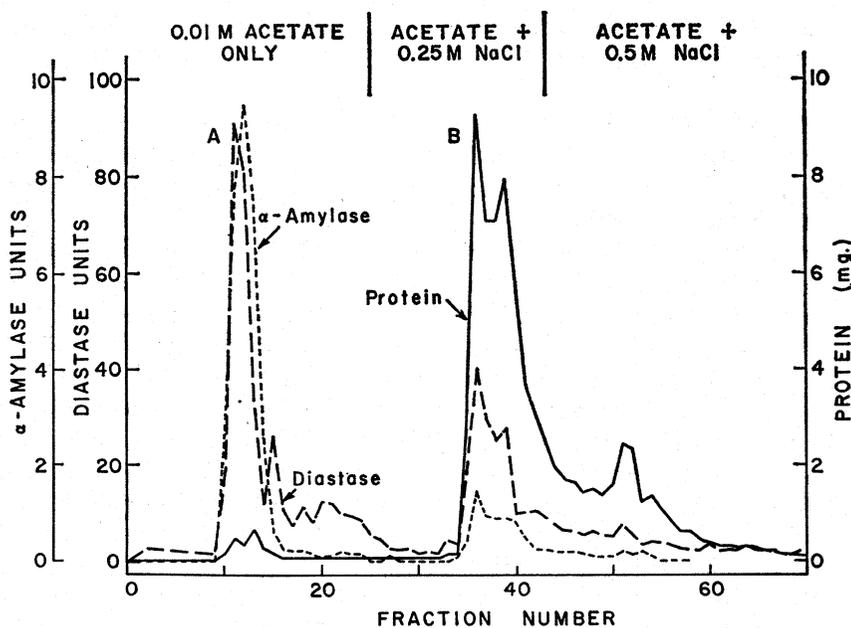


FIG. 1. Separation of honey amylase activities on DEAE-cellulose. Starting material was 6.5 ml. of concentrated enzyme solution (see text). Column packing dimensions were 23 × 1.3 cm. Development was begun with 0.01-M sodium acetate, pH 5, and continued with batchwise additions of NaCl as indicated. Fractions collected were 2 ml. each. Diastase — — —; α -amylase - - - - -; protein ———

See appropriate references for explanation of enzyme units.

4°C. The precipitate that formed was removed by centrifugation as above. The supernatant fluid (26 ml.) was made 0.6 saturated with ammonium sulphate at 4°C., centrifuged for 1 hr. at 1800 r.p.m., the precipitate redissolved in water and dialysed against water again. The final volume of this solution was 7.5 ml. A portion (6.5 ml.) of this was placed on a DEAE-cellulose column (prepared according to Sober *et al.*, 1956) and fractionated as shown in Fig. 1. The column was developed with acetate buffer (pH 5.0) followed by increasing concentrations of NaCl in the same buffer. Fractions of 2 ml. volume were collected and analysed for diastase, α -amylase and protein. The result was a resolution of diastase activity into two major bands, A and B. The relative proportions of α -amylase and diastase in these two bands indicated that in A the diastase activity was principally due to an α -amylase, whereas in B there was considerable other amylase activity, presumably due to a β -amylase. Thus it appeared that band A was mainly α -amylase, and band B a mixture of α - and β -amylases. From the protein analysis we see that band A was fairly pure, with high enzyme activity and low protein. Band B, on the contrary, contained the bulk of the protein, most of which was extraneous. Analytical data on the entire procedure are summarized in Table 1. It is evident from this table that, although losses in total enzymatic activity were considerable, DEAE-cellulose band A represented a 200-fold purification of α -amylase over the original honey. This procedure was repeated on another preparation with essentially the same results.

TABLE 1. Summary of analytical data on the preparation of amylase from honey

Preparation stage	Total diastase units*	Total α -amylase units*	Protein (mg.)	Diastase mg. protein	α -Amylase mg. protein	Diastase α -amylase
Original honey (200 g.)	6520	220.0	3180.0	2.0	0.07	29.6
Dialysed solution	6797	229.0	1663.0	4.1	0.14	29.7
Acetone precipitate	4590	148.0	731.0	6.3	0.20	31.0
Amm. sulph. precipitate	1853	59.0	134.0	13.8	0.44	31.4
DEAE bands:						
A	292	31.0	2.2	130.0	13.80	9.4
B	247	8.2	66.2	3.7	0.12	30.1

* See appropriate references for explanation of enzyme units.

Constituent fractions of bands A and B were pooled, and reaction patterns studied using starch, amylose, amylopectin and limit dextrin as substrates. The products were chromatographed on paper by the method of Albon and Gross (1952). The results were inconclusive owing to the action of a contaminant α -glucosidase. Further purification of these protein solutions was precluded by their instability as evidenced by rapid deterioration of enzymatic activity. Other methods must be developed for this purpose.

Although, in the present circumstances, we were unable to carry this work further, we did learn the following:

1. Honey diastase activity could be separated into two major components on DEAE-cellulose. One of these was principally an α -amylase, the other a mixture of α - and β -amylases.
2. Honey amylases probably differ from amylases of other sources.

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