Comparison of Digestive α-Amylases from Two Species of Spiders (Tegenaria atrica and Cupiennius salei)

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Summary. 1. Polyacrylamide gel electrophoreses of digestive fluids reveal the presence of two to three amylases (molecular mass about 58,000 Dalton) in the webbuilding spider Tegenaria atrica, and three enzymes (molecular mass about 63,000 Dalton) in the hunting spider Cupiennius salei. In either case, one amylase is largely predominant.

2. The dominating enzyme of each spider can be classified as an α-amylase (E.C. 3.2.1.1) by the binding of and activation by chloride. The $K_D$ is 1.6 mM for Cl$^-$ at 30 °C. Chloride concentrations below the optimum (<10 mM) have neither effect on pH-optimum nor on $K_m$, but lower $V_{max}$.

3. Tegenaria and Cupiennius amylases show identical behaviour with respect to all kinetic parameters studied, except for temperature dependence.

4. The Michaelis constants are around 4.5 mg/ml for soluble starch and for glycogen as substrates. Values for $V_{max}$ are slightly higher with soluble starch than with glycogen. Total activity is in the order of 50 to 75 nkat reducing groups per mg of protein of the unfractionated digestive fluid. pH-optima are near the pH of the digestive fluid (pH 7.4).

5. Discontinuous Arrhenius plots show that both amylases exist in at least two temperature-dependent conformational states. Activation enthalpy values are about 30% lower in Tegenaria than in Cupiennius amylases.

6. The amylases appear to be calcium-dependent enzymes which are non-competitively inhibited by Hg$^{2+}$ and Cu$^{2+}$.

7. Other anions activate the amylases similar to chloride, the effect decreasing with increasing ion-diameter. Fluoride does neither activate nor inhibit.

Introduction

The capability of digesting polysaccharides like starch or glycogen usually is weakly developed in carnivorous invertebrates (Barnard, 1973). Yet recent evidence showed that α-amylase was among the most active of all hydrolytic enzymes found in the digestive fluid which a spider (Tegenaria atrica) pours over its prey (Mommsen, 1978). Although α-amylases (E.C. 3.2.1.1, 1,4-α-D-glucan glucanohydrolase) have been studied in a great variety of arthropods (Barnard, 1973), very little information is available on amylases in the Arachnida. Earlier investigators like Bertkau (1885), Schlottke (1936), and Pickford (1942) gave but qualitative evidence for the occurrence of α-amylatic activity in the digestive tract of spiders. Some other publications are concerned with qualitative aspects of amylase action in ticks (Barabanova, 1972; Ehrhardt and Voss, 1961).

To get further insight into the nature of the enzymes involved in the breakdown of starch and glycogen by spiders, a detailed analysis of the digestive amylase from the web-building spider Tegenaria atrica (Agelenidae) was carried out. Simultaneously, the amylases from the digestive fluid of the hunting spider Cupiennius salei (Lycosidae) were analysed, firstly to test whether high α-amylatic activity were present also in the digestive fluid of a spider from a different family, and secondly to compare "spider"-α-amylases with amylases from other sources.

Materials and Methods

T. atrica were reared in the laboratory as previously outlined (Collatz and Mommsen, 1974b). Specimens of C. salei were a generous gift from Dr. Renate Loewe, University of Munich. All spiders were kept in the laboratory under identical conditions at 22 ± 1 °C, at high relative humidity, and fed on Calliphora. Prior to
collection of digestive juice by electrical stimulation (Collatz and Mommsen, 1974a), spiders were starved for at least 4 (T. atrica) or 8 days (C. salei). Up to 15 µl of digestive fluid were obtained from each T. atrica, and up to 80 µl from each C. salei. Digestive fluid was cooled on ice immediately after collection, and assayed within 12 h. Only adult females or females in the penultimate stage were used.

Enzymic activities were determined by the method of Bernfeld (1955) using 3,5-dinitrosalicylic acid (DNS) as a colour reagent for reducing sugars, and maltose as a standard. The assay mixture consisted of 200 µl buffered substrate solution, 25 µl solution of the appropriate anion (chloride usually given as KCl at a final concentration of 20 mM in water, adjusted to the pH required), 25 µl buffer solution containing possible inhibitors, 20 µl digestive juice (diluted 1:80 in buffer). The enzyme dilutions employed gave a linear response with incubation times up to 18 min. The reaction was started by the addition of enzyme solution and run, unless otherwise indicated, for 2 to 12 min at 30 °C. It was terminated by the addition of 500 µl DNS reagent. After heating on a boiling water bath for 5 min, the samples were diluted 1:7 with water, and absorbance read in a spectrophotometer at 546 nm against appropriate blanks. Substrate solutions (soluble starch or glycogen) were prepared freshly for every assay and dialyzed against buffer for 1 h prior to use.

For all assays (except the temperature dependence series) dialyzed digestive fluid was used. One vol. of enzyme dilution was dialyzed against 200 vols. of Tris (hydroxymethyl) aminomethane-acetate buffer (Tris-Ac) (pH 7.45, 0.05 M, 20 mM KCl) for 7 h at 4 °C. Buffer was changed every hour. For the determination of pH-optima, the digestive fluid was dialyzed against 20 mM KCl only.

Polyacrylamide gel electrophoreses (PAGE) were run on slab gels in the buffered systems of Laemmli (1970), but omitting sodium dodecyl sulphate and reducing agents, and amylases were located by the method of Gabriel and Wang (1969). Molecular weights of native amylases were estimated by PAGE according to Hedrick and Smith (1968). Protein was determined using a modified biuret method (Koch and Putnam, 1971), but reducing the final volume to 450 µl.

Michaelis-Menten kinetics were followed with substrate concentrations ranging from 1.0 to 24 mg/ml (11 values). Values for K_m and V_max were calculated from a plot of S/v versus S (Segel, 1975), without weighting. This plot proved to have the smallest systematic error, as compared with unweighted Lineweaver-Burk or Hofstee linearization (Blunck and Mommsen, 1978). Data points in the figures represent the mean of triplicates from a minimum of three independent determinations from different batches of digestive fluid.

No calcium was added to the assays, as it was found that calcium does not further activate the spider enzymes (see Results); on the other hand, calcium ions interfere with the DNS-method used (Robyt and Whelan, 1965). Determination of activation kinetics was performed at optimum pH for both enzymes (see Results), and also without addition of CaCl_2. Although calcium ions were shown to stabilize α-amylases even if this ion is only loosely bound to the enzymes (Greenwood and Milne, 1968), the addition of calcium proved to be unnecessary for the spider enzymes if undialyzed digestive fluid was used.

Results

PAGE of the digestive fluids reveals up to three bands with glycogenolytic activity for T. atrica and three bands for C. salei, respectively. One band is largely predominant in either case. Determinations of molecular weights yield values of 58,000 ± 5000 (S.E.M) for all T. atrica amylases and 63,000 ± 4600 (S.E.M.) for the C. salei amylases, respectively.

The pH-optima for the dominating amylase of both spiders lie in the slightly alkaline region (Fig. 1). The optimum for T. atrica amylase was shown to be dependent of the buffer used: maximum activity is achieved between pH 7.5 and 7.8 in Universal buffer (Davies, 1959) containing citrate, borate, phosphate, Tris and KCl (●); Tris-HCl (○); identical results were obtained with Tris-Ac or phosphate buffers containing 20 mM KCl, and phosphate buffer with 1.0 mM KCl (■). For C. salei: Tris-Ac (●) with 20 mM KCl, Tris-HCl 80 mM (○), and phosphate buffer with 1.0 mM KCl (■). The arrows indicate the pH-values of the digestive fluids.