analysis. The results, however, were not satisfactory, because melittin itself is only partially digestible due to the poor solubility in the pH 8.5 digestion buffer.

The evidence of an N-terminal formylated melittin was finally established by digestion with thermolysine. This enzyme hydrolyzes peptide bonds preceding a hydrophobic amino acid residue[7]. Therefore the hydrolysis of the N-terminal glycyl-isoleucine-bond and the formation of free formyl-glycine could be expected. The enzymatic degradation was carried out in calcium ion-containing borate buffer pH 8 for 20 h. The digestion mixture was checked by paper electrophoresis at pH 5. Besides neutral and basic products one acidic component could be detected. A compound with the same anionic electrophoretic mobility was obtained also after digestion of synthetic Form-Gly-L-Ile-Gly-L-Ala-L-Val-L-Leu-OH, but not with native melittin (Figure 3).

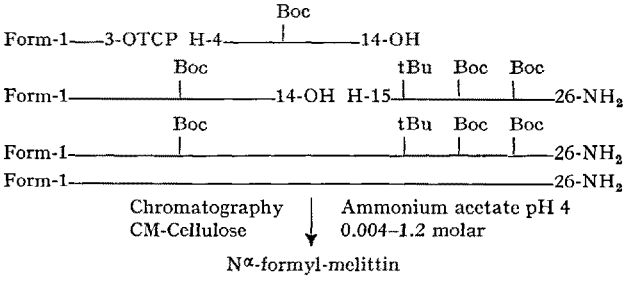

Fig. 4. Synthesis of N^{α}-formyl-melittin.

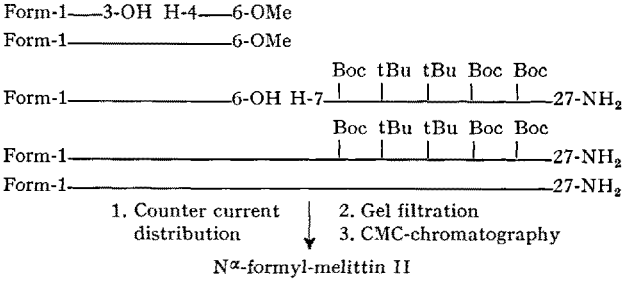

Fig. 5. Synthesis of N^{α}-formyl-melittin II.

After isolation of this product by elution from the paper and total hydrolysis only glycine was found in the amino acid analysis. The identity with formyl-glycine was confirmed by gas chromatography in comparison with an authentic sample[8].

Finally the structure of N^{α}-formyl melittin was established by synthesis[9] using in part the fragments which were used in our syntheses of the melittins[10]. The synthesis of N^{α}-formyl melittin I is summarized in Figure 4. In addition also N^{α}-formyl melittin II, which up to now has not been found in bee venom, was synthesized (Figure 5).

The synthetic N^{α}-formyl melittin has the same haemolytic activity as the native product, which is about 80% of that of melittin. Synthetic and native product are furthermore identical in paper electrophoresis, paper chromatography and in the fingerprint pattern after tryptic digestion.

Zusammenfassung. Bienengift wurde durch Gelfiltration in seine Komponenten aufgetrennt. Aus der Melittinfraktion wurde neben Melittin eine elektrophoretisch langsamer wandernde Komponente isoliert, die als N^{α}-Formyl-Melittin identifiziert wurde. Diese Struktur wurde auch durch Synthese bestätigt.

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Enzymatic Reactions in the Presence of Non-Ionic Polymers

In vitro biochemical processes are in most cases studied in aqueous buffer solutions. However biochemical reactions in vivo are proceeding in the presence of numerous low- and high-molecular weight substances whose presence influences the various chemical equilibria.

It was established previously, that antigen-antibody reactions are enhanced in the presence of non-reactive and non-ionic polymers, such as dextrans[1–8] and polyethylene glycols[9]. Since antigen-antibody reactions in many respects ressembles enzymatic reactions it was of interest to see whether enzymatic catalysis could be enhanced in the presence of water soluble non-ionic polymers. The enzyme α-amylase and a synthetic high molecular weight and water insoluble blue starch substrate[10] was used in the present work.

To the highly purified α-amylase from *B. subtilis* (Serva) a pre-incubated suspension of substrate at 37 °C was added. The reaction conditions have been described elsewhere[11,12]. The reaction was run for 30 min in the presence as well as in the absence of dextran. After the termination of hydrolysis, the coloured starch breakdown products were separated by centrifugation from the unhydrolyzed blue starch polymer and the extinction of the supernatant at 620 nm was determined using a Zeiss spectrophotometer.

Figure 1 shows the enzymatic activities obtained at different substrate concentrations in water buffer and dextran buffer solutions. As can be seen, the enzymatic catalysis performed in the presence of dextran resulted in an increased amount of product at all the concentrations of substrate used. The enhancement of the reaction may be due to the increased affinity between the enzyme and substrate. An increase in rate constant k_1 of the reaction

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

is the most likely contributory factor to the observed decrease in the apparent Michaelis constant.

The increase in the amount of product formed in standard time was found to be related to the dextran concentration (Figure 2). The effect was obtained with

as little as 1% Dextran 250 concentration. Higher Dextran 250 concentrations (2, 4 and 8%) caused a progressive increase in enzymatic catalysis. At a given dextran concentration, the enhancing effect was observed to be a function of the dextran molecular weight. The influence of 3 different dextrans, D 20, D 250 and D 2000 ($\overline{M}_w$ 2.0×10^4, 2.5×10^5 and 2.0×10^6) were tested. The highest effect was obtained with D 2000 while D 20 and D 250 gave similar but lower values than D 2000.

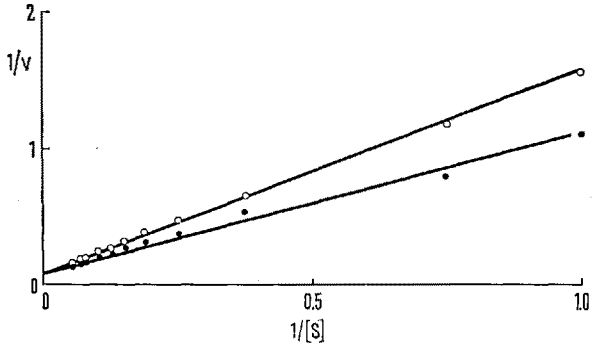

Fig. 1. Lineweaver-Burk plot of α-amylase activity throughout a 20-fold increase of substrate concentration (from 0.5–10 mg/ml of reaction mixture). The reaction was run at 37 °C for 30 min. Concentration of α-amylase was 0.1 μg/ml of reaction mixture. ●—●, in the presence of 2% Dextran 250; ○—○, in the absence of dextran.

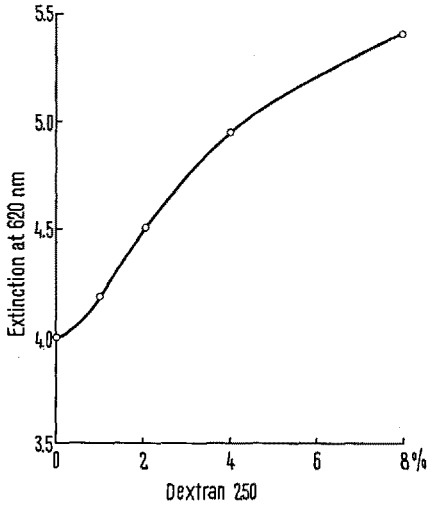

Fig. 2. Effect of dextran 250 concentration on α-amylase activity. Concentration of substrate was 10 mg/ml of reaction mixture. (Other reaction conditions as for Figure 1.)

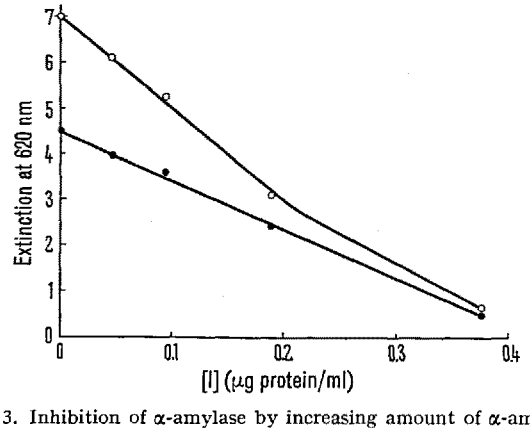

Fig. 3. Inhibition of α-amylase by increasing amount of α-amylase antibody. Substrate concentration was 20 mg/ml of reaction mixture. (Other reaction conditions as for Figure 1.) ○—○, in the presence of 2% Dextran 250; ●—●, in the absence of dextran.

It was observed previously[8], that the visible intensity of α-amylase anti α-amylase immuno-precipitates in agar gel were enhanced in the presence of dextran. It was therefore of interest to investigate whether the inhibition of enzymatic activity of α-amylase by specific rabbit anti α-amylase antibody could be enhanced in the presence of dextran. This is a unique situation in which the enzyme is allowed to interact at the same time with 2 specific macromolecules. In 2 previous separate experiments it was shown that dextran enhances the enzymatic cleavage of high molecular weight substrate (Figure 1) as well as the inhibition of α-amylase with its antibody[8]. The effect of increasing the amount of antibody in the presence and absence of Dextran 250 on α-amylase activity is seen in Figure 3. This experiment was performed at a substrate concentration of 20 mg/ml of reaction mixture. Similar results were also obtained at a lower concentration of substrate (3 mg/ml). It can be seen that in the presence of dextran the inhibition of α-amylase by the antibody is more pronounced and this effect is reflected as a higher slope of the curve. It can therefore be concluded that in this system dextran enhances the interaction between the enzyme and its macromolecular substrate; and at the same time promotes the interaction between the enzyme and its macromolecular inhibitor.

Since it was not possible to increase the enzymatic activity of an enzyme (alkaline phosphatase) which hydrolyzes low molecular weight substrate, this phenomenon may be limited to enzymes which catalyze reactions involving high molecular weight substrates or substrates known to form multiple attachments with enzymes.

The enhancement of the enzymatic catalysis is not limited to dextran. The effect was also observed with another non-ionic polymer polyethylene glycol. A comparison of apparent K_m values for several enzymatic systems in the presence of dextrans and other non-ionic polymers will be reported separately.

Zusammenfassung. Die enzymatische Spaltungsgeschwindigkeit von synthetischer hochmolekularer Stärke mit α-Amylase wird in Gegenwart von neutralen (nicht ionogenen) Polymeren erhöht. Die katalytische Wirkung hängt von der Konzentration des Polymeren und dessen Molekulargewicht ab. Die Zunahme der α-Amylase-Hemmung durch spezifische α-Amylase-Antikörper in Gegenwart von neutralem Dextran wird nachgewiesen.

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