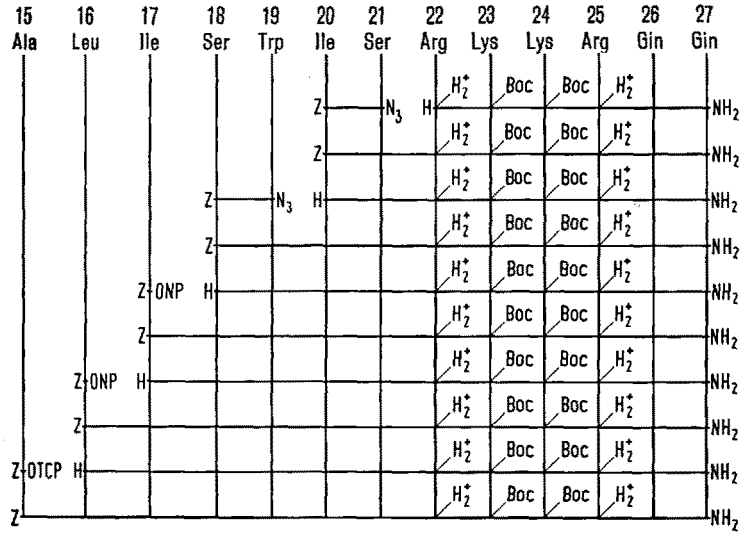

Fig. 4. Synthesis of melittin II sequence 15–27.

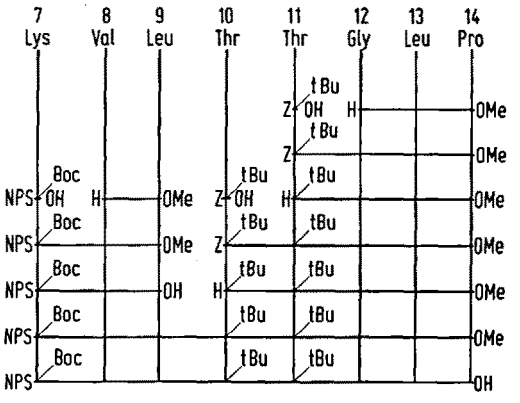

Fig. 5. Synthesis of melittin Sequence 7–14.

Haemolytic activity and action on surface tension of aqueous solutions of melittins and their partial sequences

	Haemolytic activity (%)	Surface activity (%)
1—26	100	100
1—27	100	110
7—27	3	70
Boc Boc Boc 7—23—24—27	6	120
15—26	1	5
Boc Boc 15—21—23—26	5	100
18—27	inactive	inactive
Boc Boc 18—23—24—27	–	65
Boc Boc 18—21—23—26	1	70
1—20	inactive	110
7—20	inactive	90
1—14	inactive	8
4—14	inactive	50
7—14	inactive	inactive

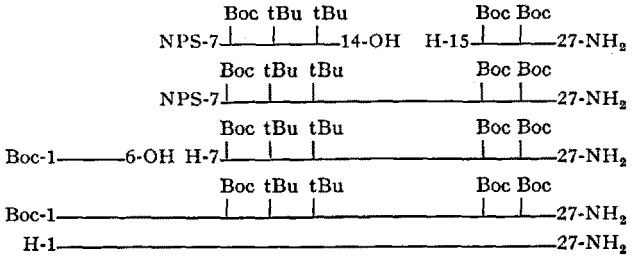

Fig. 6. Synthesis of melittin II.

of them have a strong activity on surface tension. The only intimation of a relationship between the haemolytic and the surface tension activity can be seen in the C-terminal partial sequences. Both activities are increased by blocking the N^{ε}-amino groups of the lysine residues.

Summarizing these results it must be stated that the behaviour as a typical cationic surface active compound cannot be the only explanation for the haemolytic activity, but other structural features must be of importance. Habermann and Kowallek[8], by modification of the functional groups of natural melittin, also reached the same conclusion.

Zusammenfassung. Melittin I und II wurden auf verschiedenen Wegen durch Fragmentkondensation synthetisiert. Beide Verbindungen sowie ihre Teilsequenzen wurden auf ihre hämolytische Aktivität und auf ihre Wirkung auf die Oberflächenspannung wässriger Lösungen untersucht. Die Ergebnisse zeigen, dass neben der Oberflächenaktivität noch andere Strukturmerkmale für die hämolytische Aktivität verantwortlich sind.

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D-1 Berlin 65 (Germany), 8 February 1971.

[8] E. Habermann and H. Kowallek, Z. physiol. Chem. *351*, 884 (1970).

Isolation and Structure of N^{α}-Formyl Melittin[1]

In connection with our synthesis in the melittin field, native melittin (Figure 1) was isolated from bee venom. A first separation was possible by gel filtration on Sephadex G 50 in 0.03 *M* ammonium acetate buffer pH 5.5. The effluent was recorded on a flowcell photometer at 254 nm (Figure 2). The individual tubes were pooled and the resulting fractions checked by paper electrophoresis. By comparison with an authentic sample of melittin[2] the more rapidly migrating compound in fraction 4 could be identified as melittin. The second more slowly migrating

compound in this fraction is also present in fraction 3, indicating a higher molecular weight than that of melittin. From the elution curve the elution volume for both compounds was taken and the molecular size was estimated by comparison with a standard curve. A molecular weight of about 6000 for melittin and of about 12,000 for the more slowly migrating compound was found. Surprisingly a repetition of the gel filtration with fraction 4 did not result in a separation. Both compounds were eluated simultaneously showing a molecular weight of 6000. Such a change in the status of aggregation during the isolation of melittin was also reported by HABERMANN and REIZ[3].

The separation of both compounds was possible by CMC-chromatography using an ammonium acetate gradient pH 4.004–1.2 molar. In a more efficient way melittin and the more slowly migrating compound could be isolated directly from bee venom by counter current distribution [*n*-BuOH/MeOH/H_2O/AcOH 20:5:20:1, 500 transfers, K = 0,09 (melittin) and K = 0.13 (more slowly migrating compound). In the amino acid analysis both compounds show the same composition in agreement with the proposed structure for melittin (Table).

Fig. 1. Structure of melittin. H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro Ala-Leu-Ile-Ser-Try-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2.

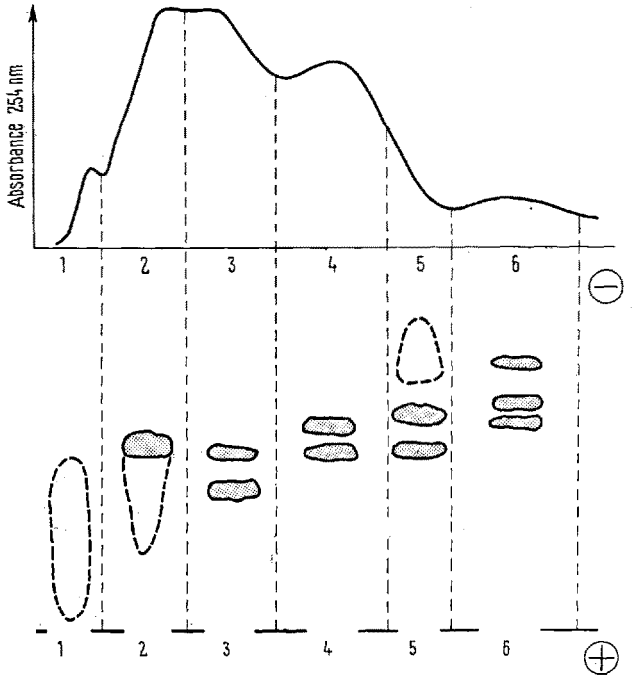

Fig. 2. Separation of bee venom into its components.

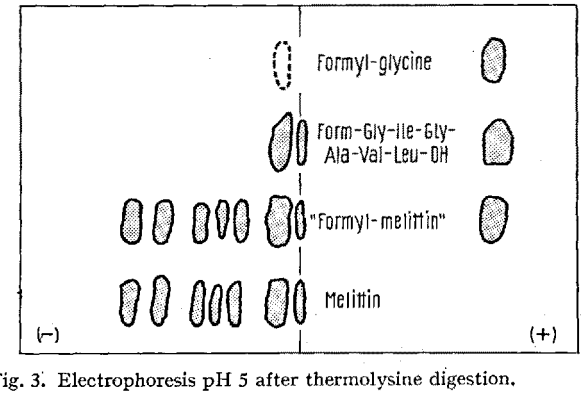

Fig. 3. Electrophoresis pH 5 after thermolysine digestion.

In this connection it must be mentioned that KREIL and KREIL-KISS[4] reported an enzymatic degradation of a crude melittin preparation. After digestion with pronase and subsequently with carboxypeptidase a ninhydrin negative compound was isolated and identified as formyl glycine. The authors assumed that besides melittin its N^{α}-formyl derivative is also present in bee venom. The isolation of this compound, however, was not described. Furthermore during the elucidation work, after tryptic digestion, HABERMANN and JENTSCH[5] isolated an N-terminal heptapeptide fragment with a blocked N-terminal amino group.

Therefore we investigated whether the more slowly migrating compound could be this N-formylated melittin. To confirm this proposed structure we attempted to deformylate this compound yielding original melittin. This reaction was carried out according to GEIGER and SIEDEL[6] using a 300-fold excess of hydrazine acetate in 65% methanol at 65 °C. After removing the excess hydrazine acetate by gel filtration on Sephadex G 25 a product with the same electrophoretic mobility of melittin was obtained. A comparison of this product with the unreacted material by tryptic digestion and fingerprinting (1. high-voltage electrophoresis in pyridine acetate buffer pH 5, 2. chromatography in *n*-butanol(pyridine)acetic acid/water 15:10:3:10) however, led to differences. In the deformylated product the spot corresponding to glutaminyl-glutamin-amide was not present, indicating a side reaction at the amide bonds. This experiment, therefore, does not prove the presence of a formyl-amino group.

In a second attempt we tried to confirm the absence of a free N-terminal amino group by digestion with leucine aminopeptidase. Incubation of melittin and the more slowly migrating compound with the enzyme was carried out at pH 8.5 and 37 °C for 15 h. After changing the pH to 3 the digestion mixture was checked by amino acid

Amino acid analysis[a] of the 2 compounds of the melittin fraction

Theory	More slowly migrating compound	More rapidly migrating compound
Gly 3	2.94	2.93
Ile 3	3.00	2.99
Ala 2	2.03	1.95
Val 2	2.12	1.99
Leu 4	3.91	4.05
Lys 3	2.95	3.17
Thr 2	1.85	1.76
Pro 1	1.16	0.76
Ser 1	0.79	0.80
Arg 2	1.82	2.01
Glu 2	1.99	2.06

[a] Values without correction for partial destruction during total hydrolysis.

[1] Peptide Synthese XLVIII. 4th Communications on melittin.
[2] We thank Prof. E. HABERMANN very kindly for sending us a sample of native melittin.
[3] E. HABERMANN and K.-G. REIZ, Biochem. Z. *341*, 451 (1965).
[4] G. KREIL and G. KREIL-KISS, Biochem. Biophys. Res. Commun. *27*, 275 (1967).
[5] E. HABERMANN and J. JENTSCH, Z. physiol. Chem. *348*, 37 (1967).
[6] R. GEIGER and W. SIEDEL, Chem. Ber. *101*, 3386 (1968).

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

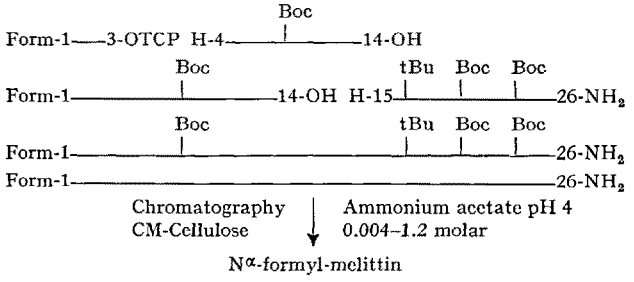

Fig. 4. Synthesis of N^{α}-formyl-melittin.

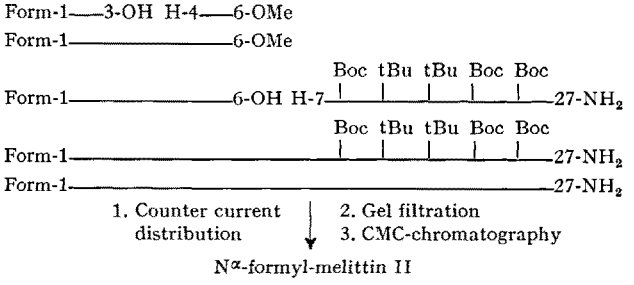

Fig. 5. Synthesis of N^{α}-formyl-melittin II.

Form-Gly-L-Ile-Gly-L-Ala-L-Val-L-Leu-OH, but not with native melittin (Figure 3).

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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[7] H. Matsubara, A. Singer, R. Sasaki and T. H. Jukes, Biochem. Biophys. Res. Commun. *21*, 242 (1968).

[8] We thank Mr. G. Baude, Section Spektrometrie und Quantenchemie (Dr. G. Hoyer) for this analysis.

[9] We thank Miss I. Beetz and Mr. M. Lehmann for the syntheses of the formylated melittins.

[10] E. Schröder, K. Lübke, M. Lehmann and I. Beetz, Experientia *27*, 764 (1971).

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