Haemolytic Activity and Action on the Surface Tension of Aqueous Solutions of Synthetic Melittins and their Derivatives[1]

Investigations of the pharmacological actions of bee venom have shown the presence of two different haemolytic principles. One of them, phospholipase A, is only indirectly active by the formation of lysolecithin from lecithin. The second principle, melittin, haemolizes erythrocytes directly and does not work with lecithin[2].

The structure of the hexacosapeptide melittin was elucidated by HABERMANN and JENTSCH[3]. During the elucidation work fragments were found which are not in agreement with the structure of melittin. Therefore, a contamination with similar peptides was assumed and a structure for one of these peptides, called melittin II, was proposed (Figure 1).

Melittin I:

1 2 3 4 5 6 7 8 9 10 11 12 13 14
H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-
15 16 17 18 19 20 21 22 23 24 25 26
Ala-Leu-Ile-Ser-Trp-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2

Melittin II:

1 2 3 4 5 6 7 8 9 10 11 12 13 14
H-Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-
15 16 17 18 19 20 21 22 23 24 25 26 27
Ala-Leu-Ile-Ser-Trp-Ile-Ser-Arg-Lys-Lys-Arg-Gln-Gln-NH_2

Fig. 1. Structure of melittin I and II.

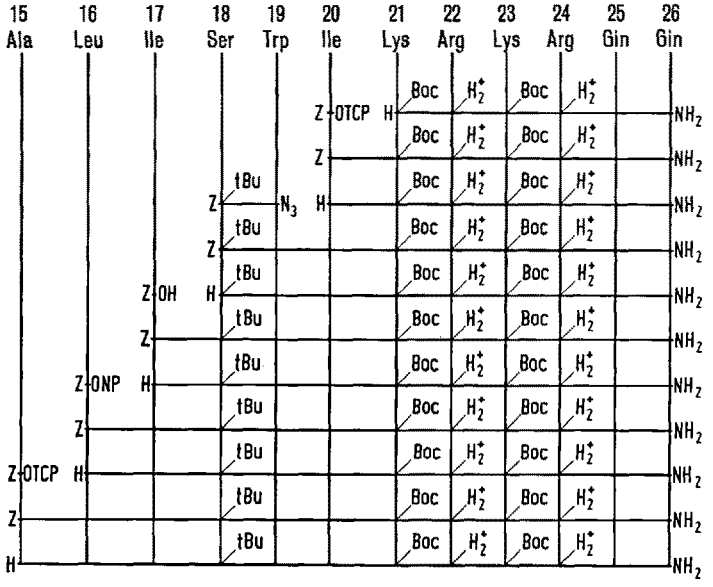
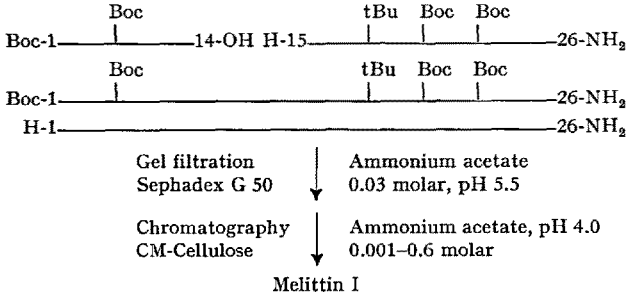


Fig. 2. Synthesis of melittin Sequence 15–26.



Amino acid analysis:

$Gly_{2.88}$, $Ile_{3.00}$, $Ala_{2.04}$, $Val_{2.04}$, $Leu_{3.90}$, $Lys_{3.00}$, $Thr_{1.76}$, $Pro_{0.97}$, $Ser_{0.86}$, $Arg_{1.78}$, $Glu_{2.00}$.

Fig. 3. Synthesis of melittin I and purification procedure.

Both melittins contain a long hydrophobic N-terminal peptide chain and a strongly hydrophilic C-terminal part. Such structures are typical for cationic surface active compounds. Therefore, beside its haemolytic activity, melittin is capable of lowering the surface tension of aqueous solutions. A comparison of the synthetic melittins as well as their partial sequences in both tests should give evidence as to what extent a correlation exists between both activities.

a) Synthesis of melittins: Melittin I was synthesized via the partial sequences 1–14 and 15–26. The synthesis of the fragment 15–26 starting with the C-terminal hexapeptide[4] is shown in Figure 2. Coupling of this dodecapeptide with the N-terminal tetradecapeptide[5] led to the protected melittin. After removal of the protecting groups by reacting with trifluoroacetic acid the purification was carried out by gel chromatography and ion exchange chromatography (Figure 3). The synthetic peptide has the full haemolytic activity of natural melittin. For 50% haemolysis 1.3 to 1.5 µg/ml of the synthetic product and 1.5 µg/ml of the natural product are necessary (calculated on the peptide content of the lyophylized material according to the amino acid analysis). For further comparative studies with native and synthetic melittin see E. HABERMANN and G. ZEUNER[6].

Melittin II was synthesized via 2 different routes. The synthesis of the C-terminal tridecapeptide 15–27 is shown in Figure 4. In the first route the sequence 1–27 was obtained by coupling the C-terminal fragment with the N-terminal tetradecapeptide 1–14, analogously to the synthesis of melittin I. In the second route the C-terminal fragment was first elongated by the sequence 7–14. The synthesis of this fragment is shown in Figure 5.

After removal of the N-terminal *o*-nitrophenylsulfenyl group the unicosapeptide was coupled with the fragment 1–6. Deblocking with trifluoroacetic acid and purification led to the pure melittin II. This synthesis is summarized in Figure 6. The synthetic melittin II shows the same haemolytic activity as natural or synthetic melittin I.

b) Structure activity relationship in the melittin field: The results of the tests of the haemolytic activity[7] as well as the action on the surface tension of aqueous solutions of the melittins and their partial sequences are compiled in the Table. In the haemolytic test, besides both melittins, only a few compounds show a small activity (sequence 7–27 and sequence 15–26). The activity of these sequences could be increased by blocking the N^{ε}-amino groups of the lysine residues in position 7, 23 and 24 as well as in position 21 and 24. All other compounds are inactive, whereas most

[1] Peptide Syntheses XLVII, 3rd Communication on melittin.
[2] W. NEUMANN and E. HABERMANN, Arch. exp. Path. Pharmak. *222*, 367 (1954).
[3] E. HABERMANN and J. JENTSCH, Z. physiol. Chem. *348*, 37 (1967).
[4] K. LÜBKE and E. SCHRÖDER, in *Peptides* (Ed. E. BEYERMANN; North Holland Publishing Company, Amsterdam 1967).
[5] K. LÜBKE, Justus Liebigs Annln Chem. *702*, 180 (1967).
[6] E. HABERMANN and G. ZEUNER, in press.
[7] We thank Mrs. E. RITTER from our Dept. Biological Chemistry (Dr. E. Gerhards) for the haemolytic tests.

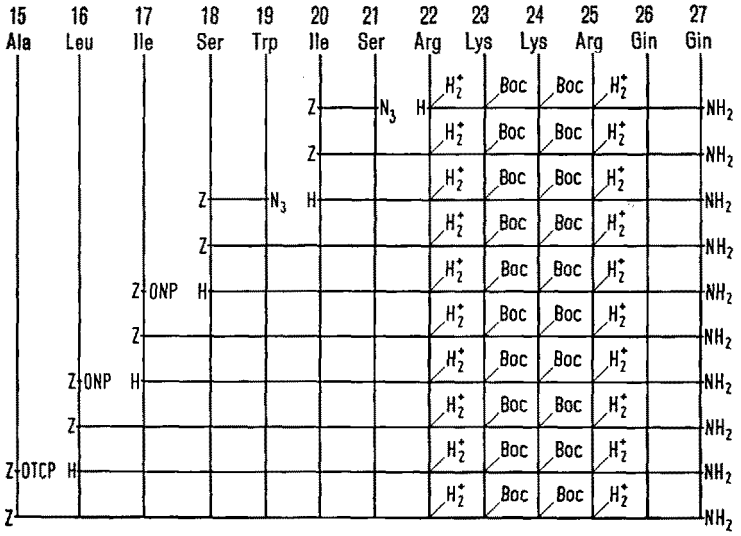


Fig. 4. Synthesis of melittin II sequence 15–27.

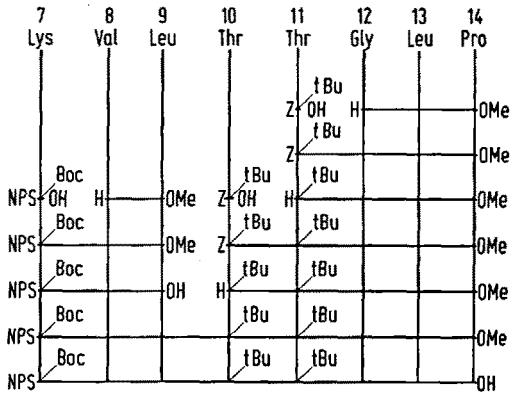


Fig. 5. Synthesis of melittin Sequence 7–14.

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

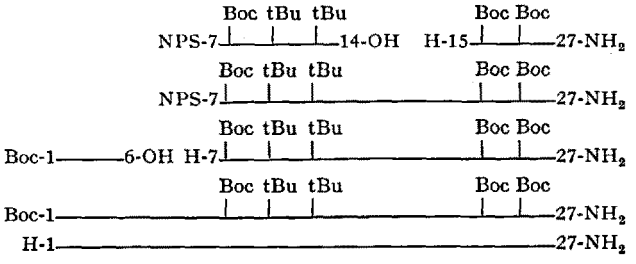


Fig. 6. Synthesis of melittin II.

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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[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