THE ISOLATION OF N-FORMYLGLYCINE FROM A POLYPEPTIDE PRESENT IN BEE VENOM

Günther Kreil and Gertrude Kreil-Kiss

Institute of Molecular Biology,[1]
Austrian Academy of Sciences and
Department of Chemistry,
University of Agriculture
Vienna, Austria

Received March 24, 1967

The main component of the venom of the honey bee, Apis mellifica, is a strongly hemolytic polypeptide termed melittin. It contains 26 amino acids starting with glycine at the N-terminus (Kreil-Kiss, 1965). The complete amino acid sequence of melittin has recently been published (Habermann et al.,1967).

In this communication the isolation and characterization of N-formylglycine from enzymic digests of melittin is reported. Approximately 10% of melittin, as isolated from lyophilized bee venom, has been found to be formylated at the N-terminus.

Materials and Methods

Lyophilized bee venom was obtained from Firma Mack (Illertissen, Germany). Melittin was isolated as described (Kreil-Kiss, 1965) or by chromatography of bee venom on a Sephadex G-75 column(1.9 x 100 cm) with 0.15 M ammonia and rechromatography of the melittin fraction under the same conditions. The purity of the preparations was checked by the absence of histidine, which is present in all the main components of bee

[1]Mailing adress: Wasagasse 9, A-1090 Vienna

venom except melittin (Fischer and Dörfel, 1953; Habermann and Reiz, 1965). Under the conditions used less than 0.01 micromoles of histidine per micromole of melittin would have been detected with the Pauly reagent. For proteolytic digestions the following commercial enzymes were used: Pronase (Calbiochem, B grade), carboxypeptidase A (Worthington, 2 x cryst.), pepsin (Nutritional Biochem. Corp., 3 x cryst.). N-acetylglycine and pyroglutamic acid were obtained from Sigma Chem. Co., N-formylglycine was synthesized according to Fischer and Warburg (1905). Melittin was digested at 36°C by incubation with 5 % (w/w) of the respective enzyme. The pH was maintained at 7.5 to 8.5 in case of pronase and carboxypeptidase A and at 1.5 to 2.5 in case of pepsin. Paper electrophoresis was performed on a Pherograph (Hormuth and Vetter, Heidelberg, Germany) in acetic acid/formic acid buffer at pH 2.0 or in pyridine/acetic acid buffers at pH 4.8 and 6.5. Total hydrolysis was accomplished by heating samples at 100°C for 2 hours in 3 N HCl. The N-acylamino acids are ninhydrin negative but can be detected with a glucose - aniline spray (1g glucose and 1g aniline in 25ml butanol/ethanol/water, 3/1/1, v/v/v). After heating the sprayed paper to 140°C non-volatile acids appear as dark brown spots on a tan background (Michl and Högenauer, 1959). The butanol/acetic acid/water system (4/1/5, v/v/v) was used for paper chromatography. Samples for hydrazinolysis were heated with anhydrous hydrazine to 100°C for 2 hours. The formylhydrazide was identified by chromatography in pyridine/aniline/water (9/1/4, v/v/v) (Narita, 1958).

Results and Discussion

The following experiments led to the isolation and identi-

fication of N-formylglycine from enzymic hydrolysates of melittin. After digestion of the polypeptide with pronase for 18 hours and subsequently with carboxypeptidase A for 6 hours, the reaction mixture was fractionated by paper electrophoresis at pH 2.0. Under these conditions all amino acids and oligopeptides move towards the cathode, while the N-acyl-derivatives of monoamino acids stay at the origin. An aliquot of the material eluted from the origin was hydrolysed in 3 N HCl and analysed for free amino acids. Only glycine and glutamic acid could be detected. The remaining portion of the eluate was further resolved by paper electrophoresis at pH 4.8. After glucose - aniline staining two ninhydrin negative components were observed, both moving further to the anode than free aspartic acid (see figure 1).

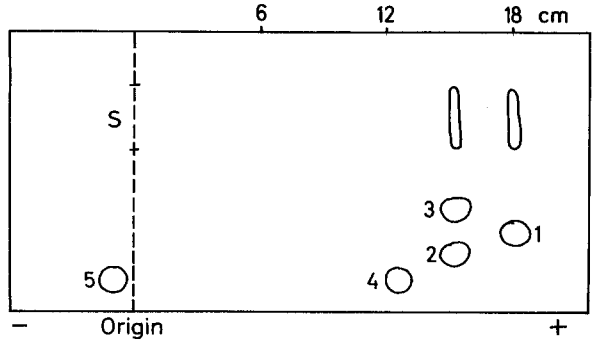

Figure 1. Separation by electrophoresis at pH 4.8 of the material (S) eluted from the origin of the pH 2.0 electrophoresis of melittin digested with pronase and carboxypeptidase A. Reference substances: 1) N-formyl-glycine, 2) N-acetyl-glycine, 3) pyroglutamic acid, 4) aspartic acid, 5) glycine.

One was identified as pyroglutamic acid, which presumably was formed through cyclization of glutamine present in the digest. The other component comigrated with synthetic N-formylglycine and yielded only free glycine after acid hydrolysis.

The glycine derivative isolated from melittin was also indistinguishable from synthetic N-formylglycine on paper electrophoresis at pH 6.5 and on paper chromatography in butanol/acetic acid/water. N-acetylglycine, which was used as another reference substance, separates from N-formylglycine under all these conditions. The above chromatographic procedure has previously been shown to separate formylglycine not only from acetylglycine, but also from propionyl-, lactyl- and benzoylglycine (Tuppy and Kreil, 1962).

Further prove of the identity of the glycine derivative as N-formylglycine was obtained through hydrazinolysis. After cleavage with anhydrous hydrazine, formylhydrazide was identified by chromatography in pyridine/aniline/water as described by Narita (1958).

In order to rule out possible artifacts, a number of control experiments were performed. To exclude the formation of formylglycine from free glycine and the formic acid present in the pH 2 buffer, this electrophoretic separation was omitted. Instead, the pronase - carboxypeptidase A digest was fractionated at 0°C on a Dowex 50-X2 column (2 x 5 cm) in 0.1 N acetic acid, adjusted to pH 2.6 with HCl. Formylglycine and pyroglutamic acid are not retained by the resin under these conditions. Small, but significant amounts of N-formylglycine were also observed after digestion of melittin with pepsin and subsequently with carboxypeptidase A or after hydrolysis with pronase alone. If the latter digest was separated by paper electrophoresis at pH 2.0, a total hydrolysate of the eluate from the origin contained also isoleucine in amounts comparable to glycine. Further incubation of the eluate with carboxypeptidase A yielded formyl-

glycine and free isoleucine. The amino acid adjacent to N-terminal glycine has been reported to be isoleucine (Habermann and Jentsch, 1967).

To estimate the amount of N-formylglycine present in melittin, the substance was isolated from the enzymic digest, hydrolysed with HCl and the glycine released quantitatively determined with ninhydrin. In one experiment 0.12 micromoles of formylglycine per micromole of melittin was found. With unfractionated bee venom the yield was 0.16 micromoles, assuming that it contains 50 % of melittin by weight. In most experiments the amount of glycine found after acid hydrolysis of the isolated formylglycine was estimated by comparing the intensity of the ninhydrin spot with spots obtained from known quantities of glycine. Yields of 0.05 to 0.1 micromoles of formylglycine per micromole of melittin were observed. These values are certainly to be judged as lower limits, since each sample has to be carried through a number of separation and elution steps where losses are almost unavoidable, and, in addition, crude pronase preparations as the one used in this study have been reported to contain deacylase activity (Webster et al., 1966).

From our results we have concluded that melittin, as isolated from lyophilized bee venom, is partially formylated at the N-terminus. To our knowledge this is the first instance that a formylated amino acid has been found in a polypeptide synthesized by a multicellular organism. The possible presence of N-formylglycine in t-RNA preparations from E.coli has been reported (Yegian et al., 1966). Since the detection of N-formylmethionine in E.coli (Marcker and Sanger, 1964) and the demonstration of its role as chain initiator in

protein biosynthesis (Adams and Capecchi, 1966; Webster et al., 1966) considerable interest in formylated amino acids has evolved. A similar role of N-formylglycine in melittin biosynthesis seems possible. Venom glands of honey bees incorporate ^{14}C-amino acids into protein and into melittin in vitro (Kreil-Kiss and Kreil, unpublished results). Using this system, the role of N-formylglycine in melittin biosynthesis should be amenable to further experimentation.

References

Adams, J.M. and Capecchi, M.R., Proc. Natl. Acad. Sci. 55 147 (1966).
Fischer, E. and Warburg, O., Chem. Ber. 38, 3997 (1905).
Fischer, F.G. and Dörfel, H., Biochem. Z. 324, 465 (1953).
Habermann, E. and Jentsch, J., Z. Physiol. Chem. 348, 37, (1967).
Habermann, E. and Reiz, K.G., Biochem. Z. 343, 192 (1965).
Kreil-Kiss, G., Monatsh. Chem. 96, 2061 (1965).
Marcker, K.A. and Sanger, F., J. Mol. Biol. 8, 835 (1964).
Michl, H. and Högenauer, G., J. Chromatog. 2, 380 (1959).
Narita, K., Biochim. Biophys. Acta 28, 184 (1958).
Tuppy, H. and Kreil, G., Monatsh. Chem. 93, 780 (1962).
Webster, R.E., Engelhardt, D.L. and Zinder, N.D., Proc. Natl. Acad. Sci. 55, 155)1966).
Yegian, C.D., Stent, G.S. and Martin, E.M., Proc. Natl. Acad. Sci. 55, 839 (1966).

Erratum

Vol. 26, No. 3 (1967), in the communication "Inhibition by Hyperbaric Oxygen of the Conversion of Cholesterol to Pregnenolone in Adrenal Mitochondria," by Peter F. Hall, pp. 320-326:

The following acknowledgment was omitted:

Acknowledgement

The author is grateful to Mr. H.A.S. van den Brenk of the Cancer Institute Board, Melbourne, for valuable advice and for the use of the pressure chamber in which these experiments were performed.