PRESENCE OF N-FORMYL- AND N-ACETYL-METHIONINE IN THE PROTEINS OF HONEY BEE THORAX

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SUMMARY: Radioactive methionine was injected into the thorax of honey bees. After several hours, mitochondrial and supernatant proteins were prepared from thorax and hydrolyzed with pronase. In these digests labelled N-formyl- and N-acetyl-methionine were detected. The latter was about evenly distributed between the two fractions, while the bulk of formyl-methionine was present in mitochondrial protein. Control experiments exclude a bacterial origin of these acyl-methionines.

INTRODUCTION: A recent addition to the growing list of features common to bacterial and mitochondrial protein biosynthesis appears to be the mechanism of chain initiation. This is mainly based on the finding that N-formyl-methionyl-tRNA, originally detected in bacteria (1), is also present in mitochondria from both yeast and mammalian cells (2,3). A dynamic role of this initiator in higher organisms is indicated by the formation of N-formyl-methionyl-puromycin in intact honey bees (4), although the subcellular site of this synthesis remains to be established. In continuation of these studies, we have chosen a more direct approach: Honey bees were allowed to incorporate ^{35}S-methionine and the thorax proteins were subsequently analyzed for the presence of acyl-methionines. Both N-formyl- and N-acetyl-methionine (f-met and ac-met) could be detected.

MATERIALS and METHODS: ^{35}S-methionine (spec. act. 300 mC/mM) obtained from the Radiochemical Centre (Amersham) was hydrolyzed in 3N HCl for 1 hour at 100° under nitrogen to cleave possible traces of acyl-

methionines. It was then dried and re-dissolved in sterile 0.8% NaCl/ 0.1% $KHCO_3$ solution. Honey bees, Apis mellifera, were obtained from local bee-keepers. About 2 µl of the solution containing ^{35}S-met were injected laterally into the thorax of each bee with a Hamilton syringe. Controls were done on bees which were killed by rapid freezing to -70° immediately after the injection. These were slowly brought back to room temperature and subsequently incubated and processed as the live bee samples. At the end of the incubation, the thoraces were dissected and gently homogenized with sand in 0.3 M sucrose - 0.01 M Tris-HCl (pH 7.2). The homogenate was centrifuged for 5 min. at 300 g to remove debris. The pellet was again ground with sand and separated at the same speed. Both supernatants were combined, an aliquot was taken for bacterial counts and the remainder centrifuged at 5000 g for 15 min. to obtain the mitochondrial fraction. This pellet was carefully suspended in 0.1 M NaOH and after several minutes the proteins were precipitated with trichloroacetic acid (TCA). From the high-speed supernatant the proteins were collected by addition of TCA. Both pellets were washed twice each with 0.5 M TCA, ethanol and ether. All equipment and media used for the isolation of mitochondrial and supernatant proteins were sterilized. The homogenate contained approximately 10^4 to 10^5 bacteria per thorax. Bacterial counts from the control samples with dead bees gave two to ten times higher values.

Mitochondrial and supernatant proteins were digested with pronase (Calbiochem) at pH 8-9 and 37°C for 24 hours. At the start of the hydrolysis one micromole of unlabelled f-met and ac-met were added. After completion of the enzymatic digestion the pH was lowered to 2.3 with HCl and the murky solution was applied to a column of Dowex 50-X2 (0.7 x 8 cm, H^+-form) previously equilibrated at the same pH. The eluate was concentrated and further resolved by paper electrophoresis at pH 4.8 (pyridine-acetate buffer). Electropherograms were cut into

sections, 1-1.5 cm wide, which were counted in toluene scintillation fluid. Peak fractions were eluted and further purified by paper chromatography in butanol:acetic acid:water (4:1:5). For the selective removal of N-formyl groups, samples were kept in 1 M HCl at 20°C for 15 hours. Fractions containing ac-met were incubated with 0.1 mg acylase I (SIGMA) in 50 mM Tris-HCl at pH 7.4 and 37°C. Methionine liberated by these treatments was identified by paper electrophoresis at pH 2 (formic-acetic acid buffer). The isolation procedure was tested by adding synthetic f-(^{35}S)-met to an unlabelled thorax homogenate. The recovery of free methionine was about 20%.

RESULTS: In a typical experiment, approximately 14 μC of ^{35}S-met were injected into each of 50 bees. One half was killed immediately after the injection by rapid freezing. Both live (L) and dead (D) bees were kept for 5 hr at 33°C. The amount of radioactivity incorporated into

Table 1. Incorporation of ^{35}S-methionine into mitochondrial and supernatant proteins and fractionation of the pronase digest. L) live bees, D) dead bees.

		Mitochondrial protein counts/min	Supernatant protein counts/min
Total radioactivity incorporated	L)	3.4×10^6	5.9×10^6
	D)	1.4×10^5	1.9×10^5
Eluate from Dowex column	L)	5.3×10^4	9.2×10^4
	D)	1.8×10^4	1.7×10^4
Formyl-methionine	L)	2300	530
	D)	110	320
Acetyl-methionine	L)	5700	12300
	D)	120	800
Microorganisms in total homogenate	L)	2.1×10^5	
	D)	8.0×10^5	

mitochondrial and supernatant proteins and recoveries during the isolation procedure are summarized in Table 1. Fractionation by paper electrophoresis at pH 4.8 of the eluate from the Dowex 50 column yielded a pattern of radioactive substances as shown in Figure 1. The bulk of the label present in the eluate of the digested mitochondrial protein was found to migrate with synthetic ac-met and a smaller peak was observed in the f-met region. Both peaks were eluted and deformylated in 1 M HCl. Of the total label migrating with f-met, 52% were converted to methionine by this treatment. Only about 3% of the radioactivity present in the ac-met region was susceptible to cleavage under these conditions. However, incubation of this material with acylase I yielded 47% free methionine. Besides small amounts of methionyl-

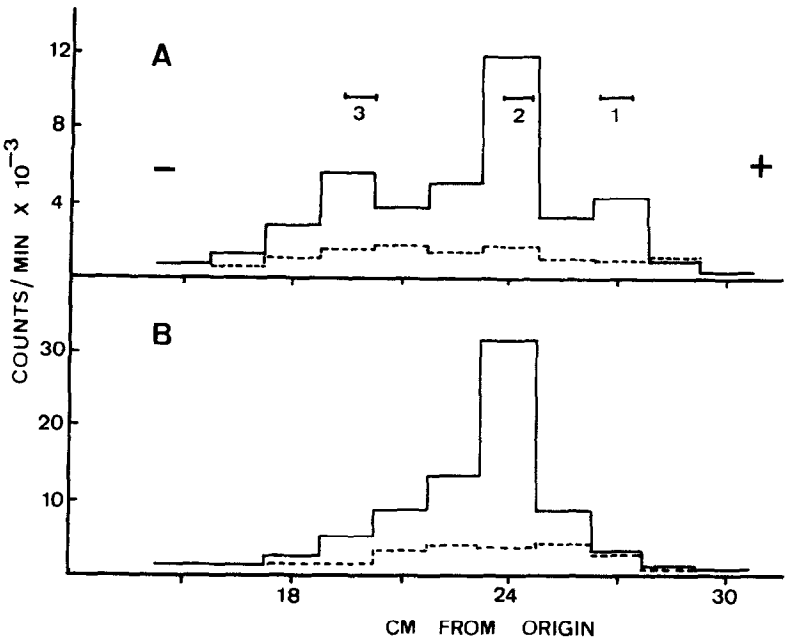

Fig. 1. High voltage electrophoresis at pH 4.8 on Whatman 3MM paper. Distribution of acidic compounds present in the pronase digest of labelled mitochondrial and supernatant proteins previously filtered through Dowex 50. Solid lines - patterns from live bees; broken lines - patterns from dead bees. Reference substances: 1) f-met, 2) ac-met, 3) picric acid, a visible marker added to the digests.

peptides, both fractions still contain labelled compounds which cannot be converted to methionine by hydrolysis in 6 M HCl at 105°C.

The digest of the supernatant proteins was fractionated by the same procedure (see Table 1 and Fig. 1B). Again, a prominent peak migrating with ac-met was observed, but considerably less f-met was present. In the control experiment with dead bees (samples marked D in Table 1 and with broken lines in Fig. 1) only traces of f-met and ac-met could be detected amounting to less than 10% of the values obtained with live bees. However, a higher number of microorganisms was found in the homogenate prepared from dead bees. This clearly shows that at least the bulk of the acyl-methionines present in bee thorax proteins is incorporated only in live insects and can not originate from contaminating bacteria.

DISCUSSION: The experiments presented in this communication show that N-formyl- and N-acetyl-methionine occurs in the proteins of bee thorax. Of these, ac-met is rather evenly distributed between mitochondrial and supernatant proteins. In the experiment outlined in Table 1, the yield of ac-met was 0.17 and 0.22% of the total methionine incorporated into the two fractions, respectively. For f-met, the corresponding figures were about 0.06 and 0.01%, the bulk being clearly present in the mitochondrial protein. If corrected for losses during the isolation, these yields would be about five times as high.

Little is known about the mode of incorporation and the metabolic role of N-acetylamino acids found at the amino end of many proteins and this also applies to proteins from bee thorax which contain ac-met. On the other hand, the function of f-met is much better understood. In bacteria, protein biosynthesis is initiated by N-formyl-methionyl-tRNA and all nascent polypeptides are considered to have f-met at the amino end. A specific deformylase subsequently hydrolyzes the N-formyl

bond (5). In Escherichia coli it has, however, been demonstrated that some proteins retain the terminal formyl group (6,7). From digests of E. coli ribosomes, f-met has been isolated (6). As shown in this paper we have obtained similar results with mitochondria from bee thorax. This complements earlier experiments on the formation of N-formyl-methionyl-puromycin in honey bees (4) and little doubt remains that mitochondrial protein biosynthesis in this insect is also initiated by N-formyl-methionyl-tRNA. This initiator has already been detected in mitochondria from other sources (2,3). The nature of the proteins synthesized by mitochondria is still not known. The presence or absence of a terminal f-met might therefore serve as a useful criterion to decide whether a protein is of mitochondrial or cytoplasmic origin.

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