AUTOMATIC DETERMINATION OF THE N-TERMINAL SEQUENCE OF CYTOCHROME *C* FROM THE INSECT *CERATITIS CAPITATA*

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1. Introduction

Comparative studies of the amino acid sequence of cytochromes *c* have provided important information regarding structure–function relationships of the protein, but, so far, only the primary structure of cytochromes *c* of a small number of invertebrates (the moth *Samia cynthia* [1], the tobacco hornworm moth [2], the fly *Drosophila melanogaster* [3, 4] and the screw-worm fly *Haematobia irritans* [4, 5] has been elucidated.

The main difficulty in isolating insect proteins is generally not the purification method per se, but rather the acquisition of enough insect starting material. It is, therefore, critical the use of the automated method of Edman and Begg [6] for sequencing an insect protein.

This report describes the sequence of the first 41 amino acids from the N-terminal end of cytochrome *c* of the dipterous *Ceratitis capitata*, determined on 300 nmoles of the protein with the aid of an automatic protein sequencer.

2. Experimental

C. capitata (Wiedemann) were bred and reared in the laboratory according to the method previously described [7]. Cytochrome *c* was isolated and purified from frozen whole adult insects by the aluminium sulfate procedure of Margoliash and Walasek [8], without crystallization.

Gel filtration on Sephadex G-50 (Pharmacia, Uppsala, Sweden) as well as rechromatography on Amberlite CG-50 (BDH Chemicals Ltd., Poole, England), pH 7.2, yielded a single peak. The protein was also homogeneous on polyacrylamide gel electrophoresis according to the method of Johns [9]. The ratio E_{550} (reduced)/E_{280} (oxidized) was 1 and the ratio E_{416} (reduced)/E_{550} (reduced) was 4.7 for the material used to determine the sequence.

The heme group was removed as described by Ramshaw et al. [10]. The N-terminal sequence analyses were carried out on an automatic (Beckman Model 890 B) protein sequencer using a procedure similar to that of Edman and Begg [6]. All reagents for the Beckman sequencer were of Sequencer Grade (Beckman Instruments, Inc., Palo Alto, California).

About 0.3 μmoles of cytochrome *c* were applied to the sequencer. The 2-anilino-5-thiazolinone derivatives were converted into phenyl-thiohydantoines by incubation in 1 N HCl at 80°C for 10 min. The PTH-amino acid residues were identified on a Beckman GC-65 gas chromatograph on SP-400 columns before and after silylation [11] and by two-dimensional TLC on polyamide layers (Cheng-Chin Trading Co., Taipei, Taiwan) [12, 13]. Arginine and histidine derivatives were identified by their specific coloration with the phenanthrenequinone [14] and the Pauly [15] reagents, respectively. Discrimination between PTH-isoleucine and PTH-leucine was achieved by the method of Edman and Sjöqvist [16] on starched paper impregnated with formamide.

3. Results and discussion

Fig. 1 shows the proposed N-terminal amino acid sequence of the cytochrome *c* from *C. capitata*.

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 -4                1                5                10
-Gly-Val-Pro-Ala-Gly-Asp-Val-Glu-Lys-Gly-Lys-Lys-Leu-Phe-Val-

          15                20                25
-Gln-Arg-Cys-Ala-Gln-Cys-His-Thr-Val-Glu-Ala-Gly-Gly-Lys-His-
          └──HEME──┘

                        35   37
-Lys-Val-Gly-Pro-Asn-Leu-His-Gly-Leu-Ile-Gly-
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Fig. 1. NH_2-terminal sequence of cytochrome *c* of *Ceratitis capitata* (numeration according to vertebrate cytochrome *c* sequences).

It has been shown that the sequences of the eukaryotic cytochrome *c* appear to be homologous to the prokaryotic cytochromes *c*; thus it is an homologous character throughout the nonviral kingdoms [17, 18]. As far as we know, insects are the only invertebrates in which the amino acid sequence of cytochrome *c* has been examined; two of them are Lepidoptera [1, 2] and the other two insects are Diptera [3–5]. Sequences of cytochrome *c* of both Lepidoptera exhibit differences in five of the amino acids, whereas cytochromes *c* of Diptera show only two positions where differences in the sequence occur (the positions 9 and 36).

The sequence of the fragment of first 41 residues of cytochrome *c* of *C. capitata* is identical to that of cytochrome *c* of *Drosophila melanogaster*. Thus, we confirm the statement that cytochrome *c* changes too slowly to be of much use in understanding the relationships among closely related organisms.

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