Purification of the enzymes of ecdysteroid 3-epimerization from the midgut of the cotton leafworm, Spodoptera littoralis.

JIAN-HUA CHEN, ROY POWLS and HUW H. REES

Department of Biochemistry, University of Liverpool,
P.O.Box 147, Liverpool L69 3BX, U.K.

During insect development, the ecdysteroid (moulting hormone) titre exhibits mandatory distinct peaks at particular times. A number of reactions may contribute to the metabolic inactivation of ecdysteroids [1]. In Lepidopteran midgut cytosol, in particular, these include ecdysone oxidase-catalysed conversion of ecdysone into 3-dehydroecdysone, which is irreversibly reduced by 3-dehydroecdysone 3α-reductase to 3-epiecdysone [2-4]. 3-Dehydroecdysone 3β-reductase also present in the midgut reduces 3-dehydroecdysone back to ecdysone [4, 5] (Fig. 1). The physiological significance of the latter transformation is unclear. The activities of these enzymes in midgut cytosol undergo distinct changes during the last larval instar of the cotton leafworm, Spodoptera littoralis [6].

As part of our studies to investigate the molecular basis of these developmental changes, the enzymes of ecdysteroid 3-epimerization from the midgut of S. littoralis have been purified and partially sequenced to allow design of primers for isolation of the corresponding cDNAs. The purification procedure involved sequential fractionation by DEAE-Sepharose CL-6B, chelation affinity chromatography, Mono-Q, Phenyl Superose and hydroxylapatite chromatography, and finally by electrophoresis (SDS/PAGE and native PAGE). This achieved 1,100-fold purification for ecdysone oxidase and the enzyme was found to be homogeneous as revealed by silver-staining on SDS/PAGE (Fig. 2a).

The native molecular weight of ecdysone oxidase was 190 kDa by gel-filtration chromatography and the subunit molecular weight was 64 kDa by SDS/PAGE. These results suggest that native ecdysone oxidase exists as a trimer. The N-terminal amino acid sequences of the intact protein and an internal fragment generated by trypsin digestion have been determined.

At least two distinct forms of 3-dehydroecdysone 3α-reductase were observed during the purification. They showed different binding properties in the ion-exchange chromatography on both DEAE-Sepharose CL-6B and Mono-Q; one was eluted with weak salt and the other with stronger salt. The native molecular weight of the first eluted 3-dehydroecdysone 3α-reductase was 50 kDa by gel-filtration chromatography. The first eluted 3-dehydroecdysone 3α-reductase preparation was also separated by native PAGE, the proteins eluted and both assayed for enzymic activity and analysed by SDS/PAGE. Comparison of these profiles indicated that this 3α-reductase was a 48 kDa protein. These combined results suggest that the native 3α-reductase may be a monomer. The second 3-dehydroecdysone 3α-reductase showed native molecular weight of 76 kDa in gel-filtration chromatography and a subunit molecular weight of 26 kDa in SDS/PAGE (Fig. 2b), suggesting that this enzyme might be a trimer. It is significant that multiple forms of 3-dehydroecdysone 3-reductases have also been detected in midgut cytosol from the tobacco hornworm, Manduca sexta [7]. Since the N-terminus of the 26 kDa subunit is blocked, we have undertaken N-terminal sequencing of peptides fractionated by HPLC following protease digestion. Direct N-terminal amino acid sequencing of the 48 kDa 3α-reductase protein was undertaken.

No apparent homologies was found to any known protein sequences, suggesting that these are novel proteins. These sequences are being used to isolate cDNAs encoding the respective enzymes.

We thank The Leverhulme Trust for funding this work.