Is C-26 hydroxylation an evolutionarily conserved steroid inactivation mechanism?

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ABSTRACT Sterols are essential components of virtually all higher eukaryotic organisms, though the exact identity of the dominating sterol varies between species, from the C-27 of cholesterol in vertebrates to the C-28 and C-29 sterols of plants and invertebrates. In addition to their role as structural components of cell membranes these sterols are also converted into a variety of biologically active hormones. This conversion generally involves modifications of the basic structure of the sterol by dealkylation, hydroxylation and/or isomerization. Recent studies have demonstrated that irreversible inactivation of both plant and insect hormones is achieved by a specific C-26 hydroxylation. The concept of sterol deactivation by 26-hydroxylation appears to be an example of an evolutionarily conserved mechanism that has persisted despite the widely varying requirements for sterols in the species where it has been detected.—Meaney, S. Is C-26 hydroxylation an evolutionarily conserved steroid inactivation mechanism? FASEB J. 19, 1220–1224 (2005)

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Sterols represent a major tool by which eukaryotic cells can refine the properties of their membranes, an ability that is considered an important step in the evolution of higher eukaryotes (1). Although the C-27 sterol cholesterol is the best known of these sterols, other sterols are often quantitatively more important in nonvertebrate species; C-28 and C-29 sterols predominate in plants and invertebrates. The sources of these sterols vary widely, however, with plants and vertebrates capable of de novo synthesis (2) whereas invertebrates such as crustaceans and insects typically require an exogenous source of sterol, at least during certain stages of life (3, 4).

While their importance for the modulation of membrane properties varies between species, sterols invariably act as precursors for different hormones that play essential roles in coordination of development, a key to the survival of any multicellular organism (5). Conversion of a sterol into an active hormone typically requires substantial modification of the parent sterol by processes, including hydroxylation, ω-oxidation, and possible isomerization. This leads to the formation of a steroid that can transduce an appropriate signal by interacting with its cognate receptor, which may be a classical nuclear hormone receptor such as the estrogen receptor or a membrane receptor such as the brassinosteroid receptor (6, 7). As a result of their potent biological activity, it is of paramount importance for an organism to control the active levels of these hormones, and sophisticated mechanisms have evolved to deactivate and subsequently eliminate them (8). Due to the presence of numerous hydroxyl and keto groups, these hormones are suitable for such (phase II type) modifications as glucuronidation and sulfonation. This situation is exemplified by mammalian steroid hormones such as estrogen and testosterone, which are excreted as sulfate and/or glucuronide conjugates.

STEREOCHEMISTRY OF THE C-26 HYDROXYLATION

Sterol 26-hydroxylation corresponds to hydroxylation of one of the two terminal methyl groups of the isopropyl steroid side chain, and may occur in mitochondrial and/or the microsomal fractions of cellular homogenates (9). Due to the nonequivalency of the terminal methyl groups of the sterol side chain, this hydroxylation creates an asymmetric carbon at C-25 and is stereospecific. Thus, hydroxylation at C-26 can lead to the formation of two stereoisomers (25R,26-hydroxycholesterol and 25S,26-hydroxycholesterol, respectively) (10) (see Fig. 1). According to present nomenclature, the methyl group that will generate the 25R-isomer when hydroxylated is denoted C-27, and that which will generate the 25S-isomer C-26 (10, 11). At present, information about the natural stereochemistry is only available from certain plants and mammals. In all mammalian systems identified to date, the majority of the 26-hydroxylation leads to the formation of 25R,26-hydroxycholesterol and occurs in the mitochondrial system (9). In contrast, 26-hydroxybrassinolide is present in plants almost exclusively as the 25S isomer.
Plants can produce many different brassinosteroids. HYDROXYLATION

A POTTED HISTORY OF STEROL SIDE CHAIN HYDROXYLATION

The main route of inactivation of both of these ecdysteroids appears to be conversion into 26-hydroxylated metabolites, and ultimately to the corresponding ecdysonic acids (24). While this has not been detailed at the molecular level, it is highly likely that a mitochondrial cytochrome P450 is largely responsible for this conversion. In an attempt to identify candidate 26-hydroxylases in insects the D. melanogaster genome was queried with representative plant and vertebrate 26-hydroxylases (CYP734A1 and CYP27A1, respectively). The sequences identified by these queries were then aligned (using Clustal 1.83) with a selection of known 26-hydroxylases from different species (see Fig. 2). Although comparative sequence analysis of P450s must be interpreted with caution, this analysis has revealed several candidate genes that are suitable for subsequent investigation by an expression cloning approach.

The situation in other invertebrates is less well explored. The cholesterol requirements of C. elegans are well known, and current evidence points to its role as substrate for hormone formation, although the identity of the cholesterol-derived hormone has remained elusive (25). These investigations are hampered by the vanishingly low concentrations of cholesterol (and the sterol-derived hormone) required by C. elegans for normal development. Recently, some clues have emerged about the identity of this steroid. In an elegant study Matyash et al. demonstrated that a sterol-derived hormone (with a polarity significantly greater than that of cholesterol) is a necessary factor for reproduction and prevention of dauer larva formation in C. elegans (26). Circumstantial evidence was presented in this
work suggesting 26-hydroxylation to be an inactivating modification, as 26-hydroxycholesterol was incapable of functionally replacing cholesterol in the experimental systems used in this study (ibid). Moreover the very recent development of a transgenic strain of C. elegans capable of converting 7-dehydrocholesterol into cholesterol (27) may facilitate definition of the steroid-derived hormones in nematodes by classical metabolic strategies.

In vertebrates steroid hormones such as testosterone and estrogen are formed after cleavage of the sterol side chain, which precludes further oxidative metabolism of the side chain. However, active side chain hydroxylation of cholesterol occurs in many tissues, most notably in connection with bile acid biosynthesis, leading to the formation of 25R,26-hydroxycholesterol and its corresponding carboxylic acid (see Fig. 3C) (28, 29). This mechanism is an important part of bile acid biosynthesis and may therefore be viewed as an exception to the general contention that this type of hydroxylation is a inactivation step. It is important to realize that this mechanism is of importance in maintaining cholesterol homeostasis and from this point of view represents an “inactivation,” though of a structural sterol rather than of a hormone. Hydroxylation of cholesterol renders the sterol metabolically unavailable to a cell and designates it for elimination from the organism (30). As this mechanism may not be absolutely dependent on the availability of apolipoproteins it may represent a more primitive mechanism for elimination of cellular cholesterol. Moreover as 25R,26-hydroxycholesterol may function as a ligand for the LXR family of nuclear receptors, its production may serve to amplify other lipid removal pathways (31).

IS C-26 HYDROXYLATION AN EVOLUTIONARILY CONSERVED STEROID INACTIVATION MECHANISM?

The presence of a capacity to generate C-26 hydroxylated sterols is apparently ubiquitous, with 26-hydroxylation typically activities present all hitherto studied phyla of plantae and animalia. The preserved site of modification of the sterols from very different organisms indicates considerable evolutionary pressure to preserve this mechanism, possibly due to an important role in inactivation.
As all of the enzymes capable of this type of hydroxylation identified to date are members of the cytochrome P450 superfamily, it is tempting to speculate that the persistence of C-26 hydroxylation is due to a requirement for a capacity to metabolize potentially dangerous exogenous sterols. For example, it has been suggested that ingestion of material rich in CYP734A1 could lead to a depletion of functional ecdysteroids in phytophagous insects, possibly preventing molting after feeding (16). It has also been suggested that phytosteroids may provide a defense against parasitic nematodes (32). An analogous situation could exist in omnivores, where ingestion of sufficient quantities of active steroids could lead to deleterious abnormalities in the absence of a suitable inactivation mechanism. One of the most patent examples of this is the capacity of CYP27A1 to rapidly metabolize 7-oxocholesterol, which is a major dietary oxysterol implicated in atherogenesis (33–35). A possible implication of these observations is that C-26 hydroxylation may have been co-opted by other synthetic pathways (i.e., bile acid biosynthesis) as the capacity for de novo synthesis of cholesterol (and the need to eliminate it) emerged in multicellular organisms. In this regard it is intriguing that C-26 hydroxylated bile alcohols represent the most primitive sterol based intestinal detergents, and that a terminal oxygen function is a necessary modification in all pathways involved in the degradation of cholesterol into bile acids (36, 37).

Further support for possible evolutionary relationships would be provided by identification of invertebrate sterol 26-hydroxylases, followed by detailed examination of substrate specificities from different species, and their potential for multiple hydroxylations of their primary substrates. Finally, it is possible to explore the capacity of hydroxylases from different species to functionally replace one another using genetically modified organisms, for example, the Cyp27a1null mouse (38) and other mutants.

Research into the evolutionary history of sterols, once the subject of keen interest (36, 39), has been reinvigorated in recent years. Significant progress has been made in elucidation of the molecular mechanisms of sterol utilization in different species, and this field promises to unearth fascinating discoveries in the coming years.

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