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Subcellular Localization of Aminosterol Biosynthesis In Squaliform Shark Liver

Squalamine is a naturally occurring aminosterol that was first isolated from spiny dogfish shark (*Squalus acanthias*). The compound is found most abundantly in the liver of this shark in the concentration of 10 $\mu\text{g/g}$ of liver tissue.^{1,2} Squalamine has potent antimicrobial and anti-angiogenesis activity; it is currently under investigation as an anticancer drug in clinical trials.³

In our laboratory, we are studying the biosynthetic pathway of squalamine and related aminosterols. Our goal is to establish that squalamine is produced endogenously and to determine whether other cartilaginous fish also produce squalamine biosynthetically. This will ultimately allow us to better understand the biological function of aminosterols in sharks.

Crude homogenates (CH) of liver were subjected to differential centrifugation to yield major subcellular fractions. These fractions were then extracted using our mini-scale extraction scheme pioneered by Shu and Musich⁴ to enrich for amphipathic compounds. The extracts were tested for antimicrobial activity by analyzing clearing zones on lawns of Gram negative, Gram positive, or fungal species.

Only weak antimicrobial activity was observed in the crude homogenate from spiny dogfish liver, suggesting low specific activity. The antimicrobial activity was enriched in all of the major subcellular fractions in spiny dogfish: nuclear pellet fractions (NP1 and NP2); the lysosome, peroxisome, and mitochondrial fraction (LMP); the total membrane fraction (TM); and the gray fat fraction (GF). The clear oil was devoid of antimicrobial activity. Other species of the Squaliform order of sharks showed antimicrobial activity in certain subcellular membrane fractions.

The squalamine-like activity associated with all of the membranes typically involved in sterol biosynthesis in liver indicates that aminosterol biosynthesis in squaliform sharks is endogenous. Livers from the other cartilaginous fish tested, including the orders Rajiformes, Lamniformes, Carcharhiniformes, and Chimaeriformes, do not show antimicrobial activity comparable to subcellular distribution of aminosterols in squaliform sharks. Aminosterol biosynthesis does not appear to be universal among all cartilaginous fish, but is active in only certain shark clads.

¹ Moore *et al* "Squalamine: An aminosterol antibiotic from the shark" *Biochemistry* 90 (1993); 1354-1358

² Rao *et al* "Aminosterols from the Dogfish Shark *Squalus acanthias*" *Journal of Natural Products* 63 (200); 631-635

³ www.genaera.com

⁴ Shu BC '01 and Musich BC '02 were HHMI interns