Commentary

Decoding the Promiscuous Activity of Bile Salt Hydrolase

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The recently identified bile salt hydrolase (BSH) from gastrointestinal bacteria catalyzes the formation of bacterial bile acid amidates (BBAAs), significantly impacting host metabolism. While this activity was characterized as promiscuous, the underlying mechanism was not explored. This commentary proposes that BSH exhibits condition promiscuity, where typical hydrolytic enzymes catalyze synthetic reactions under specific conditions. Drawing parallels with micellar enzymology, we suggest that bile salts, acting as both substrates and micelle-forming agents, create an environment conducive for BSH to catalyze amidation. This represents a potential first in vivo demonstration of such a mechanism. Future investigations should explore BSH-catalyzed reactions with bile salts below critical micelle concentrations and alternative surfactants to validate this hypothesis.

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The recently published article by Rimal *et al*. (2024) identifies a bile salt hydrolase (BSH) from the bacteria present in the gastrointestinal tract as the catalyst for the formation of the bacterial bile acid amidates (BBAAs).[1] The authors also pointed out that these BBAAs are physiologically important compounds as they affect the host metabolism.[1] Hence, this conjugation reaction merits further attention. Although the authors rightly pointed out that this is a promiscuous activity of the hydrolase, they have not made any comments about how this promiscuous activity is enabled. We suggest here a possible mechanism for it.

Protein promiscuity is used in two different contexts.[2] First are the protein-protein interactions enabled by intrinsic disorder, which are very common in regulatory proteins.[3] Protein promiscuity shown by many enzymes, on the other hand, refers to their ability to catalyze a reaction other than the one

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indicated by their EC number. Hult and Burgland[4] offered a useful classification for these latter kind of promiscuities, which include substrate promiscuity, catalytic promiscuity, and condition promiscuity. The last refers to the reverse reactions catalyzed by hydrolases under low water conditions.[5][6] The promiscuous activity observed by Rimal *et al*.[1] is an example of condition promiscuity, as a hydrolase has catalyzed a conjugation; i.e., synthetic reaction rather than hydrolysis. While catalytic promiscuous activity involves the active site residues participating differently from the normal reaction, same active site residues are involved in condition promiscuity in an identical manner. This is in line with "The N-acyltransferase activity of BSH closely mirrored the deconjugation activity with each mutation" of the active site residues.[1]

Till recently, condition promiscuity was observed, when the enzymatic reaction was carried out in a largely non-aqueous medium, such as nearly anhydrous organic solvents or ionic liquids, or deep eutectic solvents, or reverse micelles.[5] However, condition promiscuity has been now reported in quite a few biocatalytic reactions in the presence of micelles formed in aqueous medium.[7][8][9] Specifically, the most relevant example to the reported case of the promiscuous activity of the hydrolase is the esterification catalyzed by four common lipases in water in the presence of a surfactant.[10] It has been stated that the size of micelles produced by the surfactant is crucial, with micelles in the range of 50-60 nm occupying the "sweet spot".[10]

Bile salts are well-known surfactants. In view of these examples of the micellar enzymology, there is a strong possibility that the conjugation reaction observed by Rimal *et al*.[1] is perhaps the first reaction reported *in vivo*, which follows similar mechanism. Here, bile salts have a dual role, they act as substrates, and they also form the micelles, which form the necessary environment for BSHs to catalyze the formation of the BBAAs.

It may be interesting to carry out the BSH-catalyzed reaction in the solutions containing bile salts below their critical micelle concentrations and also to use surfactants other than bile salts so that the micelles are formed by the surfactants other than the substrate bile salts.

References

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Declarations

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