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Membrane properties that shape the evolution of membrane enzymes

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Spectacular recent progress in structural biology has led to determination of the structures of many integral membrane enzymes that catalyze reactions in which at least one substrate also is membrane bound. A pattern of results seems to be emerging in which the active site chemistry of these enzymes is usually found to be analogous to what is observed for water soluble enzymes catalyzing the same reaction types. However, in light of the chemical, structural, and physical complexity of cellular membranes plus the presence of transmembrane gradients and potentials, these enzymes may be subject to membrane-specific regulatory mechanisms that are only now beginning to be uncovered. We review the membrane-specific environmental traits that shape the evolution of membrane-embedded biocatalysts.

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Introduction

In the early 1950s Frank Westheimer, William Jencks, and others built on the inspired work of Maud Menten, Leonor Michaelis and other early pioneers of biocatalysis to usher in the classical era of mechanistic enzymology in which the tools of physical organic chemistry were applied to elucidate the chemical and structural basis for enzyme rate acceleration. A high water mark of this era was the mid-1970s introduction of 'perfect enzyme theory' by Albery and Knowles, which offered a rigorous conceptual and quantitative reckoning of the energetic hurdles that confront the evolution of enzymes [1,2]. They defined the nature of the energy landscapes in enzyme reaction pathways that, if attained through the process of natural selection, leads to 'catalytic perfection'. For a 'perfect' enzyme the rate-limiting step of the overall enzyme reaction at physiological substrate concentrations is diffusion of the substrate(s) to the active site. Once this condition is satisfied there is no selective pressure to further evolve the reaction chemistry, substrate affinity, or product release. Understandably, classical enzymology was devoted mainly to the study of water soluble enzymes that catalyze reactions involving soluble substrates. This opinion is devoted to integral membrane enzymes of biosynthesis, metabolism, and proteolysis that catalyze reactions for which at least one of the substrates is also membrane-associated. The focus is on integral membrane enzymes that function autonomously (rather than as part of multi-protein complexes) and that are not coupled to transport. We also glance at a couple of unusual enzymes where a water soluble catalytic domain is tethered to an integral membrane regulatory domain. We respectfully apologize to our colleagues whose voluminous work on enzymes of the respiratory chain, photosynthesis, and various types of ATPases is not treated herein because of limitations in scope and space.

What unique environmental restraints confront the evolution of membrane enzymes? We offer here a very brief survey, which complements another recent review on membrane biocatalysis. This topic seems timely in light of the remarkable progress in membrane protein structure biology over the past decade, such that there are now more than a mere handful of membrane enzyme structures available (see Stephen White's well-maintained compilation: http://blanco.biomol.uci.edu/mpstruc/). Indeed, the structural biology of membrane enzymes seems, in a great many cases, to have by-passed our knowledge of their mechanisms [3•]. In writing this opinion we acknowledge with admiration that the road from water soluble to integral membrane enzymes is bridged by an impressive body of work on the phospholipases and other soluble enzymes that bind to the membrane surface to execute interfacial catalysis (cf. [4–9]). An especially important development from those studies was the recognition that enzyme reactions on or in membranes generally conform to the Michaelis–Menten model provided that the concentrations of the enzyme and substrate are treated using membrane mole fraction or related *surface* concentration units rather than bulk molarity [10].

Membrane enzymes function within a fabulously heterogeneous medium

Integral membrane enzymes can only be removed from the bilayer when it is dissolved. A given biological

membrane will contain many dozens, if not hundreds, of chemically distinct lipid molecules [11,12]. This heterogeneity is amplified for the case of eukaryotic membrane enzymes that traffic through more than one organelle, each with a distinct lipid composition. Biological membranes are also asymmetric: the lipid composition on the outer leaflet of a bilayer does not match that on the inner leaflet [11,12]. As is the case for all membrane proteins, membrane enzymes must to some degree be structurally and functionally *tolerant* of such lipid compositional heterogeneity (see review [13]). At the same time specific protein–lipid interactions may commonly be exploited by evolution as the basis for regulating enzymes. Long-running studies [14–18] of protein–lipid interactions ranging from fleeting solvent-like contacts to stoichiometric complex formation are currently being transformed by mass spectrometry-based approaches for detecting and quantitating specific lipid–protein interactions [19•,20].

Lipids and other small molecules in the membrane sometimes play a direct coenzyme role in membrane enzyme chemistry

Membrane-associated small molecules can serve as coenyzmes for membrane enzymes. A key step in chromophore regeneration in the rhodopsin photocycle of vision is the conversion of all-*trans* 11-retinol back into 11-*cis*-retinol [21]. Lecithin retinol acyl transferase (LRAT) uses the stored energy of the ester linkage in phosphatidylcholine as the source of the energy that drives this otherwise energetically uphill trans-to-cis double bond isomerization reaction [21,22]. Another example is provided by the lipophilic ubiquinone coenzyme Q, which is used to shuttle electrons across the membrane in various reactions and pathways. These include the reaction in which electrons originating from disulfide bond formation in the periplasm of Gram negative bacteria are transferred from the periplasmic DsbA protein to membrane-embedded DsbB and thence to the freely membrane-diffusible coenzyme Q [23–25]. A similar reaction is catalyzed in the endoplasmic reticulum (ER) membrane by vitamin K epoxide reductase as part of the vitamin K cycle, which is essential for blood coagulation [26–28]. A final example is the exotic isoprenoid lipid dolichol phosphate, with its long polyprenyl tail. This lipid serves as the membrane-anchored covalent scaffold on which oligosaccharides destined for attachment to N-linked glycoproteins are synthesized. The initial reactions to add sugars to the scaffolded glycoside occur on the cytosolic face of the ER membrane. The dolichol/oligosaccharide conjugate is then actively flip-flopped to the luminal face of the membrane, followed by additional reactions to complete biosynthesis of the complex glycoside. This is followed by transferral of the fully elaborated glycoside from the dolichol phosphate head group to the asparagine side chains of nascent N-glycoproteins [29]. The cycle is completed when dolichol phosphate flip-flops back across the membrane for reuse as a scaffold. Undecaprenol phosphate serves an analogous function in related pan-membrane biosynthetic pathways in microbes, such as peptidoglycan biosynthesis [29,30].

Specific lipids are also thought to sometimes play allosteric cofactor roles in regulating membrane enzyme activity (cf. [31,32]).

Diffusion of membrane enzymes and substrates is quasi-two dimensional, but there are caveats

In an ideal fluid mosaic membrane, proteins bob up and down in the membrane plane and execute rapid axial rotation around their long axes (review in [33]). They typically also undergo lateral 2-D Brownian diffusion in the membrane plane [34,35]. Bulk membrane phases usually approximate the fluid liquid-disordered phase of ideal bilayers, although the effective viscosity of the membrane is higher than aqueous solution [34,36]. In terms of dictating the rate at which two solutes will bump into each other, the drag of increased viscosity is offset by the reduced dimensionality of the bilayer [34,37,38] such that there is no reason, *a priori*, to suppose that the rate at which a small molecule substrate in the membrane reaches a membrane enzyme is very different than for a corresponding substrate/enzyme pair in solution.

It is now appreciated that while the fluid mosaic model may apply to a significant fraction of the total area of any real biological membrane, this model is not uniformly applicable across the whole membrane [36,39,40], particularly for the plasma membrane of multicellular organisms. Some enzymes may be associated reversibly or irreversibly with the membrane cytoskeleton and are thereby fixed in the membrane. Even for free molecules, diffusion of both membrane enzymes and substrates may be transiently impeded by barriers or fences in the membrane imposed by the cytoskeleton, connections to the extracellular matrix, tight junctions, large membrane protein complexes, or other fixed molecular assemblies [36,39–41]. Moreover, as noted by Donald Engelman, 'membranes are more mosaic than fluid' [42]: as is also the case for the cytosol they too represent a protein-crowded milieu. Finally, while the term and actual manifestation of 'lipid rafts' in cellular membranes remain controversial [43–46], there seems no doubt that the plasma membranes of many cells contain transient nanodomains composed of certain lipids (particularly cholesterol and sphingolipids and proteins (usually palmitoylated) that do not diffuse or mix freely in the 2-D plane of the membrane. These transient bilayer domains are generally thought to exhibit properties that resemble the liquid-ordered phase, which has been well-characterized in synthetic lipid vesicles [47–49]. If resident in membrane nanodomains, membrane enzymes and substrates