PREFACE

Interfacial enzymology is concerned with enzymes that must access their substrates from a boundary between two phases (an interface), the most common type being the lipid–water interface of biological membranes (Berg & Jain, 2002). Interfacial enzymes are distinct from noninterfacial enzymes (Gelb, Jain, Hanel, & Berg, 1995). The latter includes enzymes in the water layer that act on substrates in the water layer. One example is hexokinase, which is a water-soluble enzyme that acts on the highly water-soluble substrate glucose. Noninterfacial also include enzymes in the membrane that act on water-soluble substrates. One example is the phosphodiesterase in the visual transduction cycle that hydrolyzes cyclic GMP. These noninterfacial enzymes have in common that their active site should access substrate in the water layer even though one of the enzymes is an integral membrane protein. Presumably the active site is well exposed to the water layer even if the enzyme is bound to membranes. Noninterfacial enzymes are sensitive to the concentration of substrate in the aqueous phase (moles of substrate per volume of aqueous phase). Rate equations describing noninterfacial enzymes are composed of rate constants and aqueous phase concentrations of interacting partners (enzyme and substrate). By contrast, interfacial enzymes must access their substrates from the interface, and the rate equations that describe their action depend on rate constants and the moles of substrate per volume of interface. Among interfacial enzymes, we also include those that access their substrates from the membrane core, and in these cases the rate equation contains terms for the concentration of substrate in the membrane core.

For enzymes that act on highly water-insoluble substrates, for example, phospholipids with long fatty acyl chains or transmembrane segments of proteins, the concentration of substrate in the aqueous phase is vanishingly small, so much so that the enzyme is forced to access its substrate from the membrane phase. These are the interfacial enzymes. Strictly speaking, it is not possible to conclude that an enzyme is interfacial based on solubility arguments alone. Proof comes from the observation of processive behavior. Consider the enzyme secreted phospholipase A_2. This is a water-soluble enzyme that absorbs onto the surface of phospholipid vesicles and thus exists in a water-soluble state (E) and an interfacial state (E^*). It has been experimentally demonstrated under some conditions that enzyme prebound to vesicles of one type of phospholipid is not able to act on vesicles of a different

phospholipid added later (under conditions where there is no intervesicle transfer of phospholipids), yet if enzyme is added to a premixture of both vesicles, it acts on both types of phospholipids (Gelb et al., 1995). Enzyme bound to the first vesicle is able to remain bound to the vesicle and catalyze the hydrolysis of several phospholipids before leaving the interface. If the enzyme was acting on the trace amount of phospholipid in the water phase, this type of processive process would clearly not be possible. These results also show that binding of enzyme to the interface (E to E*) is not the same step as loading the active site of the enzyme with substrate (Michaelis complex formation, E* to E*S). If these steps were the same, release of product from the enzyme's active site would necessarily result in release of enzyme from the membrane interface to the water layer and processive behavior would not be possible. Processive behavior of interfacial enzymes has been called "scooting" by Mahendra Jain and coworkers, whereas nonprocessive behavior is called "hopping" (Jain & Berg, 1989).

An interesting dilemma results with an enzyme that acts on a substrate that has significant solubility in both the membrane and aqueous phases. One example is platelet-activating factor acetylhydrolase that cleaves the ester of a phospholipid containing a short acetyl group instead of a long-chain fatty acyl group. The enzyme is water soluble but found *in vivo* bound tightly to lipoproteins in blood. Does the enzyme access its substrate only in the aqueous phase (for example, the membrane-bound enzyme has its active site exposed mainly to the water layer) or does the enzyme access its substrate only in the lipoprotein (for example, its active site is not well exposed to the aqueous phase)? The question is nontrivial to answer because the substrate readily partitions between the aqueous and membrane phases. Thus, at equilibrium, the concentration of substrate in the membrane phase is equal to the concentration of substrate in the aqueous multiplied by the partition equilibrium constant. Also during equilibrium, variation of the concentration of substrate in one phase leads to a proportional variation of substrate in the other phase. Any steady-state rate equation that is written in terms of moles of substrate in the aqueous phase divided by the volume of the aqueous phase (conventional concentration) can be rewritten in terms of the moles of substrate in the membrane phase divided by the volume of membrane phase (interfacial concentration) times a constant. The two equations are mathematically equivalent, and it is thus impossible to design any steady-state kinetic experiment to determine which equation applies, i.e., whether the enzyme is interfacial or not. The problem has been solved for platelet-activating factor acetylhydrolase by studying the kinetics during

the presteady-state phase in which the substrate has not yet equilibrated between membrane and aqueous phases (Min et al., 1999). This study showed that the enzyme operates on platelet-activating factor in the aqueous phase and is thus not an interfacial enzyme. One may wonder whether a membrane-bound signaling receptor that is gated by a ligand that can exist in the membrane and aqueous phases interacts with ligand in the membrane phase (interfacial receptor) or the aqueous phase (noninterfacial receptor). This question has never been answered.

For interfacial enzymes that display fast turnover numbers, the catalytic cycle may be limited by the rate of exchange of substrate between substrate aggregates containing bound enzyme. For example, some secreted phospholipases A_2 display a turnover number of $\sim 100\ s^{-1}$. If the enzyme is bound to a small unilamellar vesicle containing say 500 phospholipids, substrate would become exhausted in just a few seconds. To continue, enzyme must move to a new vesicle or there must be intervesicle exchange of phospholipids, and these processes may become rate-limiting for steady-state turnover. In this case the steady-state parameters do not reflect the true catalytic properties of the enzyme but rather those of substrate and enzyme intervesicle dynamics. This problem is especially pertinent to phospholipid–detergent mixed micelles that contain only a few phospholipids per particle (Dennis, Cao, Hsu, Magrioti, & Kokotos, 2011).

For enzymes such as secreted phospholipase A_2, there are two components to their substrate specificity. The first issue is what are the structure–function relationships that determine the affinity of the enzyme for the membrane surface (E to E*). Once bound to the interface, then comes the issue of the structural requirements of the interfacial substrate for binding to the catalytic site of E* to give the Michaelis complex E*S. For example, group IIA secreted phospholipase A_2 does not appreciably bind to vesicles that lack anionic phospholipids (for example, vesicles rich in phosphatidylcholine), yet once bound to anionic phospholipid vesicles, phosphatidylcholine that may be present in the same vesicles are good substrates for the enzyme (thus, $E^* + S \rightleftharpoons E^*S$ is favorable) (Gelb et al., 1995). Often substrate specificity studies of interfacial enzymes are carried out in ways that do not allow the deconvolution of these two processes and are thus almost impossible to interpret and to extrapolate from *in vitro* to *in vivo* settings.

Inhibition of interfacial enzymes is more difficult to study than with noninterfacial enzymes (Gelb et al., 1995). It is possible for inhibitors to act through nonspecific mechanisms, for example, compounds that partition into the membrane interface and change the physical properties of the

interface in ways that promote enzyme desorption to the aqueous phase (E* to E). More interesting and useful inhibitors are those that bind specifically to the catalytic site (or other site) on the enzyme (E* + I gives E*I); these are analogous to inhibitors of noninterfacial enzymes. Early studies of phospholipase A_2 inhibitors were plagued by the common occurrence of nonspecific, membrane-perturbing inhibitors. For example, annexins were initially characterized as phospholipase A_2 inhibitors, but later studies showed that annexins form a tightly packed array on the membrane surface and simply occlude the interfacial enzyme from binding to the vesicle to access its substrate. Thus, annexins, at least *in vitro*, are inhibitors of virtually all interfacial enzymes that must undergo an E to E* transition as part of its catalytic cycle.

Volume 583 of *Methods in Enzymology* is focused on interfacial enzymes that act on lipid substrates. Methods for detecting and quantifying the binding of proteins and enzymes to membranes *in vitro* are covered in Chapters 1, 9, 10, and 11. Methods for detection of interfacial binding of proteins and enzymes to membranes in living cells are covered in Chapters 2, 4, 6, 13, and 15. Some interfacial enzymes form protein–protein complexes, and this is described in Chapters 8 and 14. Studies to evaluate the substrate specificity of interfacial enzymes on natural members are covered in Chapter 5. Novel spectroscopic studies for providing structural insight into protein–membrane binding are provided in Chapters 7, 9, 11, and 12. Methods for production of recombinant interfacial enzymes are given in Chapters 3 and 6.

Volume 584 of *Methods in Enzymology* is focused on proteolytic enzymes that access their transmembrane peptide substrates in the membrane phase (interfacial proteases). Biochemical characterization of bacterial and eukaryotic transmembrane proteases is covered in Chapters 1, 2, 4, 5, 6, 7, 10, and 15. Kinetic studies including substrate specificity and inhibition are included in Chapters 2, 4, 5, 6, 9, 11, 12, and 15. Studies that probe the structure of transmembrane proteases are given in Chapters 3, 6, 8, and 13. Production of recombinant transmembrane proteases is the specific focus of Chapters 5 and 10.

In summary, interfacial enzymes are an important subset of enzymes. Early studies were focused on membranes that act at the lipid–water interface. More recently a new class of proteases that act on transmembrane protein segments have been discovered in several organisms. Special methods are required for the characterization of interfacial enzymes including their basic features of substrate specificity and inhibition. Structural studies are challenging because the enzyme acts in an environment that is not amenable to conventional techniques for determining molecular structure.

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