Preface

The rigorous study of flavin-dependent enzymes started more than eight decades ago. In that time, much has been learned, with perhaps the most important fact being that there is much more yet to be understood. The first purified flavoenzymes were instrumental in elucidating the role of vitamins in biochemistry and prosthetic groups in enzymology. Flavoenzymes were recognized early on for catalyzing a remarkable array of redox reactions, and for bridging the two-electron processes in intermediary metabolism to the one-electron processes of respiration that supply so much biological energy. A vast amount of research has been aimed at understanding the mechanisms of canonical flavoenzymes. These efforts were especially aided by the fact that the flavin prosthetic group is a chromophore which undergoes readily detected changes in the visible/near UV region as events such as ligand binding or intermediate formation occurred in the active site. Thus the direct study of the enzyme by rapid-reaction methods was often rewarded by the detection of intermediates whose plausibility could be matched with models from bioorganic chemistry for virtuous cycles of hypothesis, experiment, and (extremely vigorous) debate. Many of the fundamental models for particular flavoenzyme reaction types were mature well before the first crystal structures trickled—and then flooded—in. With the addition of structures and site-directed mutagenesis to complement the rich information derived from kinetics, spectroscopy, and chemistry, an understanding of the canonical flavoenzymes of unparalleled depth has emerged. A key lesson from this is the humbling realization that we still do not understand the classic paradigms fully—with the embarrassment of riches in experimental approaches and data that come to a flavoenzymologist, so also comes an expanded responsibility in explaining enzyme action in more detail.

Meanwhile, biochemistry, powered by the proliferation of technologies from molecular biology, genomics, and analytical chemistry, continually uncovers new metabolic pathways and metabolites in new organisms, leading, not unsurprisingly, to the discovery of the classic flavoenzyme reaction types applied to novel substrates. More surprisingly, however, is the new flavin chemistry—new mechanisms and new intermediates—beyond the classical paradigms that continues to emerge. Flavins now appear to form catalytic N^5 carbon and oxygen adducts. Reduced flavin has been proposed to act as a strong acid catalyst, not a redox reaction at all, in isomerizing

alkenes. Isoprenylated flavin derivatives catalyze decarboxylations in reactions proposed to go through biologically rare cycloadditions. And there are more reported noncanonical activities, detected but barely studied—hydroxylations of alkanes, reductions of alkenes, halogenations and dehalogenations, photodecarboxylations and so on. This expanding repertoire shows that despite decades of study, we have not really grasped the full chemical potential of the conjugated isoalloxazine moiety of flavins.

And so it is hoped that this volume provides a resource to support the additional work needed to understand flavoenzymes. Key fundamental techniques are described that take full advantage of the flavin as a reporter group. There are also updates on classic methods from spectroscopy that should aid in probing reactivity in yet greater detail. Important aspects are also addressed in applying powerful computer methods and in the biochemistry of important emerging systems.

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