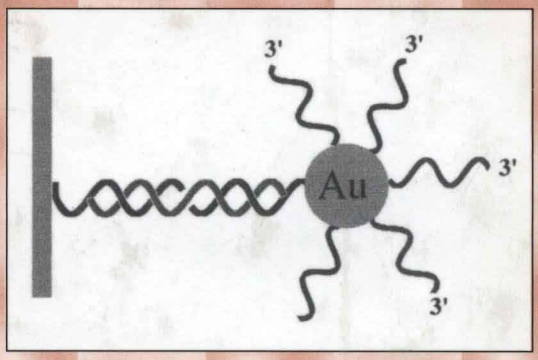
Electroanalytical Methods for Biological Materials



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Preface

These are exciting times in science! The facile electronic communication between laboratories and the increased access to information have accelerated the pace of advancement of knowledge. More than ever before, science has become a collective effort of many individuals, with common and cross purposes, in a wide variety of laboratories throughout the world. Research areas and fields intersect, and with the increased ability to communicate, cross-fertilization of ideas and methodology has become easier and more prevalent than in the past. In these times, succinct reviews of major advances in science, written by the researchers who have led the advance, become more important and useful than ever before.

This change in the way science is done and the increased pace is certainly evident in the area of science concerned with understanding electrical phenomena in biological systems. Here is a field of science that dramatically cuts across several disciplines, as reflected in the way it is referred to by its practitioners: bioelectrochemistry by electrochemists, biophysics by physicists, bioelectroanalytical chemistry by electroanalytical chemists, and so forth. It is significant that the understanding of electrical phenomena originated with things biological, in the discoveries of Galvani and Volta, more than two centuries ago. Although advances in the 19th and early 20th centuries were few, studies of electrical phenomena in nerves by Emil Du Bois-Reymond are a notable exception. Since then

this fascinating field of science has been contributing to the understanding of such diverse biological processes as energy production and DNA functions.

The impact of the advances in this field of science concerned with electrical phenomena in biological processes can be documented by the Nobel prizes awarded in this area. Among these are the prizes in medicine in 1936 to Dale and Loewi, who proved the chemical basis of neurotransmitter release; in 1963 to Hodgkin and Huxley for the sodium-potassium ion pump model of nerve impulses; in 1970 to Katz, von Euler, and Axelrod for mechanisms of humoral transmission in nerve cells; in 1978 to Mitchell for his chemiosmotic theory of the electrochemical membrane gradient that drives ATP synthesis; in 1991 to Neher and Sakmann for understanding of the function of single-ion channels; in 1997 in chemistry to Skou for discovery of the ion-transporting enzyme Na+,K+-ATPase, and continuing in 2000 in medicine, to Carlsson, Greengard, and Randel for signal transduction in the nervous system.

This timeline of accomplishment is continued in the chapters of the present compilation. Mark Wightman and Andrew Ewing have a direct link to the most important accomplishments, with their in vivo electrochemistry work described in this book. This electroanalytical approach to the real time measurement of neurotransmitters in living cells of the brain, which was pioneered in the laboratory of R. N. Adams, is a technique that works and is approaching a state of maturity. It is this kind of methodology that will allow future scientists, regardless of their mother field, to continue to unravel Nature's mysteries. In recent years charge transport along the pi stack of the DNA double helix has been demonstrated, notably in Barton's laboratory at Cal Tech, and its role in biology hotly debated. Shana Kelley, who has played a major role in the work from Barton's laboratory, reviews and puts this topic into perspective. Regardless of the importance of DNA charge transport pathways, the development of electroanalytical polynucleotide hybridization sensors promises to be a foundation of future sensing technology. Joe Wang, who has pioneered in this area, reviews various approaches and strategies for these sensors; and Willner and Katz summarize the newest results from their laboratory in Jerusalem.

The use of electroanalytical methodology has yielded significant insight into the workings of redox enzymes. An important section of the present compilation is devoted to the voltammetry of redox enzymes from the laboratories of Fraser Armstrong, Lo Gorton, Fred Hawkridge, and Jim Rusling, all major players in this area. These chapters describe the subtleties of the electrical and chemical requirements for facile communication between the redox centers of the enzymes and the electrode transducers. The power of the simple voltammetric experiment, in the hands of experts, is abundantly evident in these reviews. Especially interesting is the role of cell membrane mimic layers that point back to a fundamental understanding of how electrical information is transferred in biology.

Without the newest bioelectroanalytical methods, the solutions to problems that have to be addressed to prevent and limit human disease may be too long in coming. The chapters on bioelectroanalysis include descriptions of the most advanced and successful methods, which in large part build on the fundamentals described in the earlier chapters. The pioneering work on electrochemical immunoassays is described by its inventor, Bill Heineman. Werner Kuhr describes the future of sensor technologies, Steve Weber summarizes the strategies that work in the determinations of amino acids and peptides, many of which were developed by his group, and Jim Cox summarizes the challenges in finding and developing new catalysts for bioanalysis. The advances that have been made in complex biological determinations are apparent in the chapters by Adam Heller and his coworkers on the development of glucose and other in vivo sensors, by Bob Kennedy, and by the Lunte group, who describe powerful combinations of modern separations and newest electroanalytical methods in the analysis of extremely small and complex biological samples. These last chapters face the wide horizons of the future developments that seem so close to realization. We hope that you will find useful new information in this book, and we look forward to the new developments that the work described in this book is likely to inspire.

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Electroanalytical Methods for Biological Materials

Contents

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Charge Migration Through the DNA Double Helix

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I. INTRODUCTION

The charge-transport properties of the DNA double helix have intrigued chemists, physicists, and biologists essentially since the structural features of this molecule were revealed over 40 years ago [1]. The stack of aromatic heterocycles within the double helix allows the readout of genomic information through the display of functional groups within the grooves of this molecule. The striking similarity of the π-stacked array of DNA bases to π-stacked solid-state conductors has prompted the suggestion that DNA might efficiently facilitate charge transport [2]. This intriguing proposal, along with relevance of charge migration in DNA to biological function and biosensing, has prompted the examination of this phenomenon from many different scientific perspectives.

Given the central role of DNA in cellular function, the dynamics and distance dependence of electron-transfer reactions proceeding through this medium have important biological ramifications. Both damage to DNA bases [3,4] and repair of base lesions [5] can result from the reaction of radicals with DNA. Therefore, the extent of charge migration through DNA would determine whether only localized reactions affect the integrity of genomic information or whether chemistry initiated from a distance might also play a role in DNA damage and repair.

Moreover, with the recent progress in decoding the human genome and identifying diseases that result from genetic errors, inexpensive and accurate methods to read out DNA sequence information are needed so that genetic testing can become a more integral part of medical care. The characterization of the

charge-transport properties of DNA provides the basis for the development of a new class of biosensors. By monitoring DNA-mediated electron-transfer reactions sensitive to sequence or structural perturbations, genetic information could be extracted from DNA samples using methods that may possess advantages over conventional assays [6].

The aim of this chapter is to describe recent results that illustrate the salient features of electron-transfer reactions mediated by DNA and, in particular, the implications that these results have for DNA-based biosensing. The unique structural features of DNA strongly influence electron-transfer reactions proceeding through this medium. Moreover, structural perturbations drastically attenuate reactivity, and, in particular, disruptions in base stacking within the DNA helix significantly decrease the efficiency of electron transfer. The sensitivity of DNA-mediated electron transfer to base stacking has been exploited in the development of an electrochemical assay for point mutations that may have applications in genetic testing.

II. EVIDENCE FOR LONG-RANGE ELECTRON TRANSFER IN DNA

Less than a decade after the structural features of the DNA double helix were elucidated, the first experiments directed at understanding charge migration in DNA were initiated [2,7,8]. In 1961, inspired by the structural resemblance of DNA to conductive one-dimensional crystals, Eley and Spivey measured bulk conductivity of dry DNA and observed high levels of electron mobility [2]. These results were both contradicted and confirmed by later experiments performed by different laboratories [7,8]. In the ensuing 40 years, the extent of DNA-mediated charge migration has been studied by a variety of experimental methods, and many different conclusions concerning the efficiency of charge transfer through DNA have been drawn. For a detailed account of the history of the field of DNA-mediated electron transfer, which has developed considerably over the past 10 years, the reader is referred to other reviews [9–11].

In the past five years, a significant number of experimental studies substantiate Eley and Spivey's original claim that the DNA base stack could promote long-range charge migration [12–27]. In well-defined assemblies containing covalently constrained photoactive molecules associated with the DNA bases through either intercalation or stacking interactions, a cohesive body of experimental results supports the notion that long-range redox reactions are facilitated by the double helix of DNA. In addition, recent measurements of the electrical conductivity of DNA molecules offer another line of evidence that this material has conductive properties.

A. Photophysical Studies of DNA-Mediated Electron Transfer

The study of photoinduced electron transfer between molecular donors and acceptors provides a means to assess the electronic coupling provided by the DNA helix. Early applications of this method to DNA-mediated reactions utilized reactants noncovalently bound to DNA [28–30] These studies provided qualitative information concerning the efficiency and distance dependence of electron transfer, but the ambiguity associated with random distributions of reactants along the DNA helix precluded a quantitative analysis. Once chemical methods were developed for the covalent attachment of donors and acceptors to DNA [31], the distance dependence of these reactions, as well as the effects of perturbations within the intervening medium, could be systematically studied. The identification of unnatural DNA bases with appropriate photophysical and redox properties has also helped to define the extent of electronic coupling provided by the base stack [16].

1. Photoinduced Electron Transfer Between Intercalators

In DNA duplexes covalently derivatized with ethidium (Et), a classic organic intercalator, and $Rh(phi)_2bpy^{3+}$, a metallointercalator, reductive electron-transfer quenching (Figure 1A) was monitored as a function of distance and intervening stacking [12]. The photoexcitation of ethidium generates a singlet excited state with sufficient energy (E^o (Et^{2+}/*Et) ~ –0.9 V vs. NHE) to reduce $Rh(phi)_2bpy^{3+}$, which has a low-energy, ligand-centered reduction (E^o ($Rh(phi)_2bpy/Rh(phi)(phi^-)bpy$ = –0.03 V vs. NHE). The loss of *Et fluorescence in the presence of $Rh(phi)_2bpy^{3+}$ was measured to monitor the efficiency of electron transfer in the intercalator-modified duplexes.

A series of DNA duplexes ranging from 10 to 14 base pairs in length were prepared with the intercalators attached to opposite 5′ ends through short aliphatic linkers. Despite the long distances (17–35 Å) that separated the intercalators, fluorescence quenching occurring on a subnanosecond timescale was detected [12].

The yield of this electron-transfer reaction decreased with increasing donor-acceptor distance, but interestingly, the rate of the reaction was not greatly attenuated. Thus, the distance dependence of this quenching reaction does not solely reflect changes in electron-transfer rate and is therefore not purely a measurement of β (the decay of electronic coupling) [32]. The distance dependencies of reactions exhibiting this behavior are represented by the symbol γ. For the DNA-mediated reductive electron transfer reaction between *Et and $Rh(phi)_2bpy^{3+}$, $\gamma = 0.1$ Å^{-1}.

To test the response of this long-range electron-transfer reaction to perturbations in the intervening medium, and confirm that the pathway for the reaction

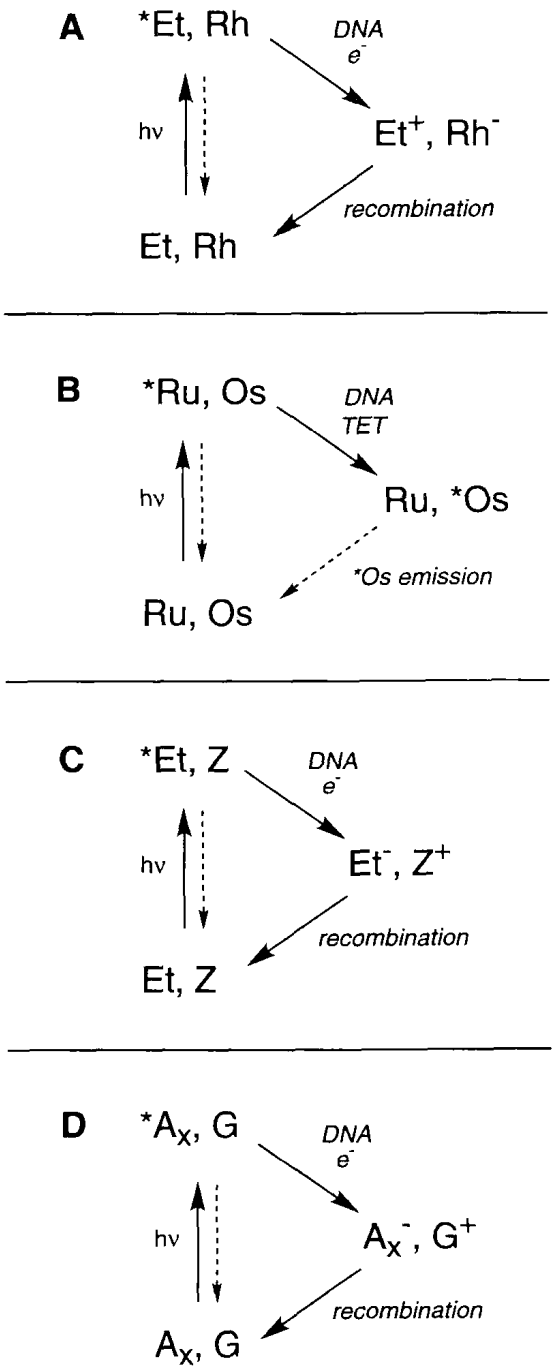

Figure 1 Reactions used to study electron transfer in DNA with photophysical methods. (A) Photoinduced reduction of $Rh(phi)_2bpy^{3+}$ by *Et [12]. (B) Triplet energy transfer (TET, a double electron transfer) from $*Ru(phen)(bpy)(Me_2\text{-}dppz)^{2+}$ to $Os(phen)(bpy)(Me_2\text{-}dppz)^{2+}$ [13]. (C) Photoinduced oxidation of Z by *Et [14–15]. (D) Photoinduced oxidation of G by adenine analogues ($*A_x$ [16]).