# Biological Events Probed by Ultrafast Laser Spectroscopy

Edited by
R. R. ALFANO

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# Preface

The development of picosecond laser technology over the past ten years has enabled scientists to investigate some of the most important primary processes occurring in nature. Fundamental information on the structure and dynamics of these photobiological processes has been obtained from direct temporal measurements on the ultrafast time scale ranging from $10^{-13}$ to $10^{-9}$ sec. In particular, the primary processes that take place in photosynthesis, vision, hemoglobin, and DNA have been investigated with picosecond time-resolved laser spectroscopy. Indeed a great wealth of information has been obtained that has enhanced our perception of nature. Knowledge about these developments may be gathered from original research contributions scattered in scientific journals. Review articles have been published from time to time, but they have usually dealt with one particular topic or phenomenon. Available textbooks on biological processes do not cover primary events in any detail. There is a definite need for a book that covers the introductory as well as the current research work on the various aspects of ultrafast phenomena in biology.

This book reviews current progress in the experimental and theoretical understanding of primary phenomena that occur in biology on a picosecond and nanosecond time scale. It is possible for the interested reader to gain a complete overview of all the important breakthroughs and developments in understanding primary events during the past ten years (prior to 1982). In this volume, the reader will find a review of basic principles, an up-to-date survey of research results, and the current thinking of experts in the fields of photosynthesis, vision, hemoglobin, and DNA.

This book should prove a useful source both for the newcomer to the field of ultrafast phenomena and for the experienced. The book was written to attract biologists, chemists, physicists, and engineers who are interested in biological processes, and it will give them the opportunity to find all the necessary and relevant material in one presentation. The high quality of the research reviewed and covered by the contributors to this volume will enlighten the beginner as well as the expert. It is my goal that this book will stimulate future research in understanding primary events in biology.

The book is organized into five parts—the primary events in the various areas of biology are reviewed in the first four parts, and picosecond and subpicosecond laser techniques are covered in Part V.

The primary events in photosynthesis are reviewed in Part I. Wong presents an introduction to the primary processes in photosynthesis. Pellegrino and Alfano review fluorescence kinetic measurements, while Ke and co-workers review absorption kinetic measurements in higher plants. Netzel reviews kinetic measurements in bacteria photosynthesis. Work on model systems is described by Katz and Hindman. The theories of primary energy transfer appropriate to photosynthesis are covered by Swenberg. Multiexcitation processes are discussed in the articles by Geacintov and Breton and by Mauzerall.

The primary visual processes are discussed in Part II. Callender presents the introductory material. Peters and Leontis review the kinetic experimental work on rhodopsin and Ebrey reviews the kinetic work on bacteriorhodopsin. The theoretical concepts and current thinking on the primary events in vision are reviewed in the chapters by Birge and by Honig.

In Part III, Eisenstein and Frauenfelder present the introductory material on the kinetic models of hemoglobin and myoglobin. Interesting picosecond measurements on hemoglobin and myoglobin are reviewed by Noe.

Measurements on DNA are reviewed by Shapiro in Part IV. Part V covers the subject of picosecond and subpicosecond pulse generation and measurement techniques.

I wish to thank the contributors for their cooperation in this endeavor and Drs. R. Callender, A. G. Doukas, N. E. Geacintov, B. I. Greene, R. Knox, A. Lamola, B. Parson, F. Pellegrino, and S. L. Shapiro for their reviews, advice, and comments. Special thanks are given to Mrs. M. Gibbs for secretarial assistance, and to Academic Press for its continued interest and cooperation. I gratefully acknowledge NSF, NIH, AFOSR, NASA, and Hamamatsu for their support over the years.

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PART I

# Photosynthesis

## CHAPTER 1

# Primary Processes of Oxygen-Evolving Photosynthesis

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## I. Introduction

Energy flow into the biosphere (the thin layer of living materials on the surface of the earth and in the upper layer of the ocean) occurs through the conversion of solar energy to chemical energy by the process of photosynthesis in higher plants, algae, and certain bacteria. In the case of green plants, the process consists of the oxidation of water to oxygen and the reduction of carbon dioxide to carbohydrate in the presence of chlorophyll and light:

$$CO_2 + H_2O \xrightarrow[\text{light}]{\text{chlorophyll}} (CH_2O) + O_2 + 112 \text{ kcal/mole} \quad (1)$$

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Historically, the progress leading to this understanding was made in the late eighteenth and early nineteenth centuries. The capacity of plants to produce free oxygen was discovered by Joseph Priestly in 1772, the need for light and chlorophyll was observed by Jan Ingen-Housz in 1779, the necessity for carbon dioxide was recognized by Jean Senebier in 1782, and the involvement of water was found by Nicolas Theodore de Saussure in 1804. The realization that carbohydrate was the organic product came about 1850. The understanding that the fundamental physical function of photosynthesis was the conversion of light energy to chemical energy was due to Julius Robert von Mayer (discoverer of the principle of conservation of energy) in 1845. Over the next century progress in the general understanding of the process was slow and often dependent on technological progress. During that time, a number of discoveries were made and concepts developed which were later to provide important foundations for what can be called the modern era of photosynthesis, which began in 1960. The reader is referred to a paper by Myers (1974), titled "Conceptual Developments in Photosynthesis, 1924–1974," for a brief account of events during that exciting period of photosynthesis research.

Since 1905 it has been known that the reactions in oxygen-evolving photosynthetic systems are of two types—those activated by light (light reactions) and those that are not directly dependent on the presence of light (dark reactions). The light reactions involve two light-induced charge separation steps connected in series by a sequence of oxidation–reduction components, forming what is called the electron transport chain. The photochemical steps are known simply as photoreactions I and II, and the sites are the reaction centers. The net result of photoreaction I is the formation of a weak oxidant, the oxidized reaction center I, and a strong reductant, the reduced primary electron acceptor of photoreaction I, while that of photoreaction II is the formation of a strong oxidant, the oxidized reaction center II, and a weak reductant, the reduced primary electron acceptor of photoreaction II. Enhancing the efficiency of light collection for the reaction centers are a battery of light-harvesting chlorophylls (known also as bulk or antenna chlorophylls) and other accessory pigments, the chemical nature of which is dependent on the biological species of the organism.

The antenna, the reaction center, the electron transport intermediates, and the structural protein and lipid components make up the photosystem (PS), the functional apparatus for the photoreactions—hence PS I and PS II. Distinguished on the basis of the reaction rates, the light reactions are divided into two groups: primary events [half-times ($t_{1/2}$), $10^{-15}$–$10^{-9}$ sec] consisting of the absorption of light, transportation of excitation energy, and induction of charge separation by the excitation energy, and secondary events ($t_{1/2}$, $10^{-8}$–$10^{-1}$ sec) consisting of events such as the movement of electrons through the electron transport chain, production of adenosine