DRUG–DNA INTERACTIONS

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STRUCTURES AND SPECTRA

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About the Cover: The cover was created by G. Strahan, and is composed of the circular dichroism (CD) spectrum and a molecular model of a complex formed when the drug Distamycin binds as a dimer to an AT-rich DNA sequence (see Chapter 3). The molecular model was generated by the program AMBER9, using the methods outlined in Chapter 1 and Appendix 2, and visualized by the program UCSF Chimera.

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The authors wish to dedicate this book to their wives,
Kimiko Nakamoto, Yo Tsuboi, and Kellie Hom.

CONTENTS

PREFACE

Drug discoveries are made serendipitously or systematically. In the latter case, a large number of derivatives of a candidate drug molecule are prepared and screened first by cytotoxicity tests *in vitro* since no definitive structure–activity relationships are available. This process is inefficient and time-consuming, in spite of the recent advent of combinatorial chemistry and laboratory automation. Hence, the ultimate goal of this book is to contribute to our knowledge of the structure–activity relationship found in drug–DNA interaction by coalescing much of the basic structural information, thereby improving the efficiency of the drug discovery process.

First, however, we must discuss the structures of drug–DNA complexes that were determined by a variety of physicochemical and biochemical techniques. Although some of these structural studies have already been reviewed by other authors, they tend to focus on structural information on the atomic level. In this book, we have emphasized global information obtained by a variety of spectroscopic techniques. It is hoped that this book will serve as a guide for students in chemistry, biochemistry, and pharmacology who are interested in learning the nature of drug–DNA interactions and as a reference book for academic and industrial researchers who are doing research in this field.

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INTRODUCTION

Cellular DNA is the major target of most antitumor drugs, as well as many antiviral and antibacterial agents. Depending on the mode of interaction, these drugs can be classified as intercalating, groove-binding, covalent bonding, and strand-breaking drugs. They can distort the double-helix structure of DNA, alkylate or cleave a DNA strand, thereby inhibiting DNA replication and transcription, which are the necessary preconditions for cell division. Previously, the nature of the drug–DNA interaction was investigated extensively using a variety of physicochemical and biochemical techniques. The results obtained from these studies have begun to shed light on the underlying principles of the structure–activity relationship, which is indispensable for understanding the mechanism of the drug–DNA interaction and for designing new and more effective drugs with fewer side effects.

Although many review articles on drug–DNA interactions (e.g., see Refs. G1–G8 listed at the end of this Introduction) have been published, comprehensive reviews combining structural and spectroscopic studies have not yet been available. The aim of this book is to provide such information using examples chosen from a large number of references. Cytotoxicity data are quoted whenever available and appropriate. It is hoped that any unbalanced presentation may be compensated by the review articles cited throughout the book. Drug–DNA interactions on the cellular and clinical levels and drug–protein interactions are not included because detailed structural and bonding information on these systems is limited by their complexity.

In this book, the terms *DNA* and *drug* are used loosely to cover a wide range of chemicals. Thus, *DNA* includes its components such as purine and pyrimidine bases, nucleosides as well as synthetic oligonucleotides, and natural DNA. Likewise, a *drug* (or a ligand) refers to any (natural and synthetic) chemical with potential pharmacological activity. Most of these are anticancer drugs, although antiviral, antibacterial drugs and a wide range of other chemicals such as biological stains and metal complexes are included.

This book focuses largely on structural and bonding information obtained by physicochemical methods, namely, X-ray crystallography, NMR spectroscopy, ESR spectroscopy, and optical spectroscopy such as UV–visible absorption, fluorescence, circular dichroism (CD), flow linear dichroism (FLD), and infrared (IR) and Raman (R) spectroscopy. Among these methods, X-ray crystallography and NMR spectroscopy are the most important when atom-level structural information is desired. However, X-ray crystallography is limited to drugs bound to relatively short oligonucleotides *in the crystalline state*, because it becomes more difficult to obtain single

crystals of diffraction quality as the nucleotide sequence becomes longer. Although multidimensional NMR spectroscopy coupled with computer-aided molecular modelling techniques can provide structural information on the atomic level *in solution*, this method is also limited to drug molecules bound to relatively short nucleotides since NMR signals tend to overlap in longer oligonucleotides. It should be noted that the molecular structure determined in the crystalline state suffers from the lattice effect, which could influence the molecular conformation. Although the solution structure obtained by NMR spectroscopy may be less precise than X-ray structure, it is closer to the structure in a biological environment and can provide insight into molecular flexibility. Furthermore, it is possible to study the effects of changing the pH, temperature, and drug–DNA mixing ratio on the drug–DNA intraction in solution and even dynamic equilibria between different conformers.

In contrast, the other spectroscopic techniques mentioned above are applicable to drug molecules complexed to long oligonucleotides such as poly(dG-dC)$_2$, poly(dA-dT)$_2$, and natural DNA, and provide structural and bonding information on the molecular level. It should be noted, however, that detailed structural information obtained by X-ray and NMR studies may not always be directly applicable to those with long oligonucleotides, because the structure of the latter is affected by the flexibility and base-pair sequence of their strands.

Since the main objective of this book is to review structural and spectroscopic information obtained by the aforementioned techniques, we provide only a very limited discussion of their basic theories and experimental methods. This information is found mainly in Chapter 1, with some expanded details presented in the appendixes. More thorough information on these topics may be found in many monographs and review articles cited (e.g., see Refs. (G9–G14).

As stated earlier, and discussed at greater length in the following chapters, the modes of the drug–DNA interaction can be classified into the categories of *intercalation, groove binding*, *covalent-bonding and strand breaking* as well as a variety of their combinations. Furthermore, their bindings are often reinforced by hydrogen bonding, Coulombic forces, and van der Waals interactions. These are discussed throughout this book, which consists of six chapters.

Chapter 1 provides basic knowledge about the structures and spectra of bases, nucleosides, and nucleotides, and is vital to the understanding of the nature of drug–DNA interactions discussed in later chapters. It explains all of the conventional nomenclatures regarding DNA structures for the reader who is not familiar with DNA structures. For more information, the reader should consult those books and review articles on DNA structures that are abundantly available. Chapter 1 also provides an overview of how this structural information can be derived from the experimental methods.

Chapter 2 deals with drugs bound to DNA *via intercalation*, which was discovered in 1961 and is the best known mode of drug–DNA binding. Acridines, aclacinomycins, actinomycins, and ethydium bromide are typical examples. More than 20 other intercalating drugs are listed together with their sequence preferences. Recently, a variety of bis and tris intercalating drugs have been prepared to attain stronger binding and better selectivity of binding sites, and their modes of binding are discussed.

Chapter 3 focuses on *groove-binding* drugs such as netropsin and distamycin, which interact with AT-rich region of DNA in the minor groove via hydrogen bonding and Coulombic and van der Waals forces. Sequence-specific drugs have been prepared by arranging pyrrole and imidazole rings in a specific order since pyrrole binds to A/T base whereas imidazole prefers to interacts with the G base. Some derivatives of netropsin and distamycin exhibit antitumor and antiviral activities much stronger than those of distamycin. Chromomycin, mithramycin, and their derivatives represent another type of *groove-binding* drugs that interact with the GC-rich region of DNA. A variety of hybrid drugs in which groove binders are connected to intercalators have been synthesized and their modes of interactions with DNA investigated.

Chapter 4 describes *covalent bonding* drugs (e.g., alkylating agents) such as (+) CC-1065, anthramycin, ecteinascidins, and mitomycins. Active-carbon atoms of these drugs form *covalent bonds* with the N3 atom of adenine or guanine, and with the N7 atom and exocyclic NH_2 (N2 amine) group of guanine. Hedamycin and atromycin B are known as *intercalating alkylators.* Although metal-containing drugs such as cisplatin and its derivatives also form *covalent bonds* with DNA, they are discussed separately in Chapter 6, because their interactions with DNA have been studied much more extensively than other covalent bonding drugs.

Chapter 5 deals with *strand-breaking* drugs. Bleomycins and enediyne antibiotics such as calicheamicins, esperamicins, and neocarzinostatins are able to cleave either single- or double-stranded DNA. Bleomycins consist of a DNA-binding domain and a metal-binding domain that are connected by a linker. The former brings the latter close to the DNA strand, while the latter, in the presence of the Fe(II) ion and O_2, produces "activated BLM," which is responsible for DNA *strand breaking.* Green BLM-Co (III)-OOH serves as an ideal model of the "activated BLM" because it is a stable, diamagnetic compound and the structure of its complex with a short oligonucleotide can be determined by NMR spectroscopy. Enediyne antibiotics contain highly strained enediyne rigns that produce phenylene biradicals in the presence of a nucleophile such as thiol. Simultaneous cleavage of both strands of DNA by these biradicals is responsible for their strong antitumor activities.

Chapter 6 covers a large number of metal-containing drugs. Among them, cisplatin is best known, and its binding to DNA has been studied most extensively. The major products of cisplatin–DNA reaction are *1,2-intrastrand crosslinking* adducts formed by *covalent bonding* of the Pt atom with the N7 atom of guanine or adenine The minor products are *1,3-instrastrand and 1,2-interstrand crosslinking* adducts between two guanine bases. Thus, the main interest of research is on *1,2-intrastrand crosslinking* adducts, particularly those involving two guanine bases. To search for more potent drugs with fewer side effects, a large number of cisplatin analogs have been synthesized, and their cytotoxicities have been tested. These include cisplatin derivatives in which its NH_3 ligands are replaced by a variety of monodentate and bidendate (chelating) ligands, and transplatin and monofunctional complexes such as $[Pt(NH_3)_3Cl]^+$. Dinuclear platinum complexes can form *1,4-interstrand crosslinks* with DNA, and some of them are highly cytotoxic and more effective against cisplatin-resistant cell lines. Other metal-containing drugs

included are those of Pt(IV), Pd(II), Au(III), Ru(II,III), Rh(III), Cu(II), and Zn(II), the cytotoxicities of which are reported.

Appendixes include discussion of several topics on Raman and NMR spectroscopy and molecular modeling that are not described in the main text of Chapter 1. This appendix material is intended to help the interested reader understand some of the more technical aspects of these methodologies.

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