Chirality in Biological Nanospaces

Reactions in Active Sites

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Dedication

Dedicated to my research students for sharing their interest in my research, and to my family members for their unstinting support and forbearance

Preface

The relationship of molecular chirality and biology is intriguing. Our curiosity prompts the question as to why diverse natural biological molecules are overwhelmingly dissymmetric. Is it so that nature uses the dissymmetry of biological molecules to create specificity in the interaction with other molecules? Does the myriad network of interaction aim to control the functionality of a bewildering variety of molecular machines with ease, fidelity, and efficiency? If so, how? Whatever the answer is, eventually the orientation-dependent interaction between molecules in the confined state in biological systems is responsible for the manifestation of many fascinating properties.

Enzymes exhibit remarkable capacities for discrimination as they carry out life processes. Despite the amazing variety of the structure and function of the enzymes, it is expected that unifying principles exist in their structural organization. Evolution must have developed, retained, and continued to further develop these minimal features for the better efficacy of a reaction. Active site structures of enzymes are nanosized spaces. Their confined geometries are designed by evolution to recognize and capture the specific substrates, place them in favored geometry, and finally drive forward the formation of the product to continue life processes. It is tempting to look into the active site structure and the discrimination therein to understand how nature utilizes the chiral structures of molecules in carrying out vital biological reactions much more efficiently than the corresponding synthetic processes. The conserved features of the organization of the active site structure of enzymes indicate that a network of electromagnetic interactions controls the remarkable fidelity of the reaction. The pattern of interaction is an outcome of the intricate interplay of electrostatic (such as charge–charge, charge–dipolar, polar–polar, induction type, and hydrogen bonding, to name a few), hydrophobic, or van der Waals interactions. The network of interaction depends on the orientation-dependent arrangement of the active site residues around the reactants, and confinement of the reactants is important here. The evolutionary processes develop the structure of the active site and network of interaction in order to perform reactions with improved speed, accuracy,

and efficiency. With this perception in view, this book aims to provide the author's perspective about the influence of chirality in driving reactions within enzymatic cavities of nanodimension.

Chirality embraces numerous biological, biomimetic, and nonbiological systems, and the topic touches an extremely broad area of phenomena. Tremendous advances are being made in chirality-related fields using principles of physics, chemistry, biology, and mathematics. Often, chirality shows its manifestation in seemingly disparate phenomena. This book is not intended to provide a compendium of the wide variety of manifestations of chiralities in different systems, and no attempt is made to be exhaustive in this respect. Enzymes being the workhorses of the cells, their workings are of high significance and study in this field is greatly advancing. A vast body of literature exists about the chiral specificity of different enzymes. Since research on the stereoselectivity in enzymatic reactions is continuously growing, an anthology of stereoselectivity in enzymatic reactions is beyond the scope of this book. Consequently, if the reader is interested in learning about a specific class of enzyme, its structure, or biochemistry related to stereoselectivity, he or she is advised to look elsewhere for that information. On the other hand, readers will gain from this book a clear view of how the interactions between the active site residues and the substrate molecules influence reactions.

The author has written a brief review on the related topic in the *International Reviews in Physical Chemistry*, and his curiosity with the subject grew into this book. It was apparent during the time of writing the review, and still now, that there is a dearth of books that concentrate exclusively on the interaction of chiral molecules within the active site structure. This book evolved to fill this gap. It is intended primarily for graduate students, teachers, and researchers in the fields of chemistry, biology, medicine, pharmacy, and related interdisciplinary academia such as nanobiology and nanochemistry. One needs only basic ideas of intermolecular interaction as applicable to biophysical chemistry to make use of this book. The theoretical developments have been described at a simple, unsophisticated level throughout. As the subject is in a state of constant evolution and a comprehensive understanding is yet to be achieved in many arenas, constructive suggestions are most welcome.

It is our hope that the views presented in this book shall be further developed to design custom-made novel biocatalysts with better efficiency. Often a trial-and-error method is employed to develop synthetic enzymes, and the process is time consuming and expensive. A combination of crystallographic studies with electronic structure based computational analysis as described in this book may lead to future elucidation of new drugs that can target biological active sites with better efficacy.

Acknowledgments

It is a pleasure to thank research students Sindrila Dutta Banik and Krishnan Thirumoorthy for sharing the author's own interest in the chiral discrimination in biological systems. It is a delight to witness their dedication in conducting research work related to chiral discrimination in biological systems in the author's laboratory.

A large part of the research work described in this book was conducted at the University of Kalyani and Birla Institute of Technology and Science–Pilani. It is a pleasure to thank the enthusiastic students at these institutions for their active participation in the learning process. Their questions and responses stimulated the author's interest in biophysical chemistry as a constantly developing subject.

The author has cherished the delight of learning from all the teachers throughout his academic career and owes a great deal to their contributions in shaping his perspective on scientific principles. It is a pleasure to thank Dieter Vollhardt and Biman Bagchi for collaborations on various aspects of chirality in biomimetic systems.

The author is immensely thankful to Sindrila Dutta Banik for her assistance regarding many aspects of the preparation of this book—checking the manuscript and references, preparing most of the artwork, as well as the cover art, to name a few. Her help has been immeasurable.

It is a great pleasure to acknowledge the support of all the family members and friends. The patience of Anwesha Nandi and Shamita Goswami at home made any hardship during the preparation of the manuscript much easier to bear. This book could not have been completed without their indulgence in allowing the author to work unusual and long hours. The author takes this opportunity to express appreciation for the encouragement received from Shibshankar Nandi, Sunity Nandi, and the late Nirendra Kisore Nandi as well.

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Finally, it is a delight to thank Lance Wobus of Taylor & Francis Group who proposed and initiated the writing of this book and waited patiently. The author also thanks Taylor and Francis Group, CRC Press and especially David Fausel, Jennifer Derima, Judith Simon, Cristina Escalante, and Andrea Grant for their assistance in the production of this book.

About the author

Nilashis Nandi was born in Cooch Behar, West Bengal, India (1965). He received his B.Sc. (Hons.) (1983) and M.Sc. (1985) degrees from North Bengal University and Ph.D. (1992) from Visva Bharati University. He became a postdoctoral fellow at the Indian Institute of Science, India (1993–1997), a J.S.P.S. postdoctoral fellow at Nagoya University, Japan (1997–1999), and an Alexander von Humboldt postdoctoral fellow at the Max Planck Institute of Colloids and Interfaces, Germany (1999–2000). Dr. Nandi was a faculty member in the chemistry group of Birla Institute of Technology and Science, Pilani, India from 2001–2007 and became a professor in the Department of Chemistry, University of Kalyani in 2008 where he has worked ever since. His research interest is focused on theoretical and computational studies in biophysical chemistry.

List of abbreviations

aaRS: Aminoacyl tRNA synthetase
aatRNA: Aminoacyl transfer RNA
ADH: Alcohol oxidoreductase
AIDS: Acquired immune deficiency syndrome
AMP: Adenosine monophosphate
ATP: Adenosine triphosphate
BactnABC: Bacteroides thetaiotaomicron chondroitinase ABC
BAM: Brewster angle microscopy
BCH: 2′-deoxy-3′-thiacytidine
BS: *Bacillus stearothermophilus*
BSSE: Basis set superposition error
CD: Circular dichroism
ChonABC: Chondroitin lyases ABC
CoM: Cofactor coenzyme M
COX: Cyclooxygenase
CS: Chondroitin sulfate
CYP: Cytochrome P450
DFT: Density functional theory
dNTP: 2′-deoxyribonucleoside 5′-triphosphates
DS: Dermatan sulfate
E. coli: *Escherichia coli*
EC: Enzyme commission
EchA: Epicholorohydrin epoxide hydrolase
EH: Epoxide hydrolase
EPR: Electron paramagnetic resonance
ER: Enoyl reductase
GIXD: Grazing incidence X-ray diffraction
GlcA: Glucuronic acid
HCN: Hydrogen cyanide
HDG: 1-O-hexadecyl glycerol
HF: Hartree-Fock
HIV: Human immunodeficiency virus
HNL: Hydroxynitrilase or hydroxynitrile lyase

HSV: *Herpes simplex* virus
IC_{50}: Half maximal inhibitory concentration
IR: Infrared
2-KPC: 2-(2-ketopropylthio) ethanesulfonate
2-KPCC: 2-ketopropyl-CoM oxidoreductase/carboxylase
K_d: Dissociation constant
LCP: Left circularly polarized
LOX: Lipoxygenases
MD: Megadalton
MM: Molecular mechanics
MP2: Møller-Plesset (perturbation theory)
NADPH: Nicotinamide adenine dinucleotide phosphate (reduced)
NMR: Nuclear magnetic resonance
NOS: Nitric oxide synthase
ONIOM: Our n-layered integrated molecular orbital and molecular mechanics
OPA: One photon absorption
PFE: *Pseudomonas fluorescense* esterase
PM3: Parameterization 3 (of MNDO)
PKS: Polyketide synthase
PPi: Pyrophosphate
PTC: Peptidyl transferase center
PvulABCI: *Proteus vulgaris* chondroitinase ABCI
QM: Quantum mechanics
RCP: Right circularly polarized
R-HPC: [(R)-2-hydroxypropylthio]-ethanesulfonate
RNA: Ribonucleic acid
RT: Reverse transcriptase
SAG: Stearyl amine glycerol
TPA: Two-photon absorption
tRNA: Transfer RNA
TT: *Thermus thermophilus*
VCD: Vibrational circular dichroism
VOA: Vibrational optical activity
Yeast: *Saccharomyces cerevisiae*
ΔE_{LL-DL}: Chiral discrimination energy

Contents

chapter one

Introduction

A casual look is sufficient to draw our attention to the presence of symmetry in many of the objects around us. Apparent symmetry is observed in the animal kingdom, plants, as well as inanimate objects. Leonardo da Vinci's drawing of Vitruvian man reflects the symmetry present in the human figure. Bilaterally symmetric animals (other than humans) are also quite abundant and are examples of symmetrical natural objects. The reflection symmetry observed in the shapes of leaves and the radial symmetry present in the shapes of sea anemones are also common examples of symmetrical organisms. Symmetry is common among inanimate material objects. The impressiveness of architectural wonders such as the Taj Mahal, the pyramids, and the Parthenon is due to their unique symmetry. Obviously, the presence of symmetry creates an aesthetically pleasing sense related to the concept of proportionality and balance as well as the beauty of the object. The presence of symmetry in material objects not only appeals to the aesthetic sense but is also of practical use. Use of symmetry has been made for safety, security, and familiarity since ancient times. Use of symmetry rather than dissymmetry in the construction of a house or a room makes it convenient and livable for the inhabitants. Identifying an object in the latter case would need more effort than in the former case. This idea can be extended to various tools of day-to-day use. The reflection symmetry of bricks is used in construction, and helical symmetry is used in the working principle of drill-bits and springs. Numerous other examples can be given that indicate a preference for symmetry (both at the simple and complex levels) both in nature as well as in artificial habitats.

Is this apparent presence of symmetry ubiquitous or not? Notably, the concept of the presence of symmetry (or its absence) is related to the length scale of the object under consideration. A close inspection of the reflection symmetry in the shapes of leaves reveals that the leaf vein patterns show imperfect bilateral symmetry at the length scale of leave veins. Natural biological systems exhibit both symmetry and dissymmetry at various length scales of their structural details. The helical structure of the polypeptide shown in Figure 1.1 is a dissymmetric object. The dissymmetry of the polypeptide helix at the microscopic length scale is due to the presence of an asymmetric carbon atom (except glycine) in each of the constituent amino acids. On the other hand, the alpha helix structure of a

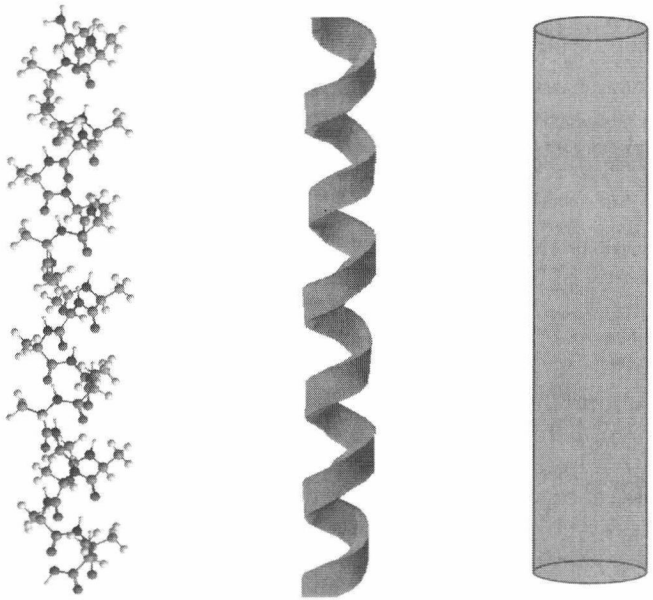

Figure 1.1 Length-scale-dependent symmetry and dissymmetry in a polypeptide chain using different levels of coarse-graining of the molecular structure. Different representations of a polypeptide are shown as (a) an all-atom representation exhibiting dissymmetry at the level of primary structure arising out of the chiral centers of individual amino acids, (b) helical representation showing the periodicity of the peptide linkage at the level of secondary structure, ignoring the atomistic structure, and (c) cylindrical representation showing the symmetry around the helix axis ignoring any underlying structural details. The dissymmetry is gradually lost in going from Figures 1.1a to 1.1c.

protein can be considered at the length scale of the complete helix as having cylindrical symmetry about its long helical axis (Figure 1.1). When the dissymmetry of the microscopic length-scale structure is ignored and the shape of the helical object as a whole is considered, the cylindrical symmetry of the object is apparent. In observing the symmetry of an object (or conversely, its absence), it is necessary to consider the relevant length scale. This length-scale dependence of symmetry is more apparent when one considers a functional biological molecule such as a protein. The possible point symmetry operations for protein quaternary structures are rotations since each subunit is a dissymmetric object, in general. The presence of the C_2 point group in α-chymotrypsin, oxyhemoglobin, and deoxyhemoglobin, the D_2 point group in concanavalin A, and the D_3 point group in insulin is recognized (Cantor and Schimmel, 1980, 127) despite

the fact that all proteins at the microscopic length scale (length scale of amino acid or helical structure) are dissymmetric objects. Lack of symmetry at a certain level of the structural hierarchy is not only typical for proteins but is observed in many other biological molecules such as nucleic acids or lipids. Biological systems are overwhelmingly dissymmetric or chiral. Macroscopic, mesoscopic, and microscopic chirality can be noted in biological systems. Fundamental biomolecules such as amino acid and sugar are chiral. These molecules principally exist in one particular enantiomeric form and are examples of chirality at the primary or microscopic level. Since structure and function in biology are intimately related, a pertinent question is whether the chiralities of these natural objects have any influence on their functionality and if so, how they are related. We discuss this issue in the following text after a brief introduction to chirality and chiral discrimination.

1.1 *Chirality and chiral discrimination*

Chirality (from the Greek word *cheir*, meaning *hand*) is an attribute of nature that is observed in its various forms. Traditionally, chirality is considered a geometric property of various natural as well as nonnatural objects that makes them nonidentical and nonsuperposable with their own mirror images. A chiral object and its mirror image are enantiomorphic. On the other hand, an achiral object is superposable on its mirror image. This broad definition of chirality includes diverse macroscopic objects such as hands, animal organs, biological organisms, crystals, and macromolecules as well as microscopic objects such as various molecules as chiral entities (Eliel et al., 1994). If the object lacks an improper or alternating axis of symmetry, including a center of symmetry and a plane of symmetry, then it is chiral (Jacques et al., 1981, 3). A necessary and sufficient condition for the chirality of a molecule is that it cannot be superimposed on its mirror image as improper rotations are regular rotations followed by a reflection in the plane perpendicular to the axis of rotation. A molecule possessing an asymmetrically substituted carbon atom (four different groups as shown in Figure 1.2a) is a common example of chiral molecules and lacks all symmetry elements (other than the identity E or C_1). According to this definition, objects of various length scales, starting from the macroscopic objects mentioned earlier, to mesoscopic objects such as small- to medium-sized aggregates of molecules, as well as molecules of microscopic dimensions could be chiral entities. Chiral molecules such as amino acid and sugar are quite common natural objects. However, as exceptions, chiral molecules without asymmetric carbon atoms exist, and examples of achiral molecules with more than one asymmetric carbon atom are also well known. A detailed account of various aspects of molecular chirality is available in the standard literature (Eliel et al., 1994;