ADVANCES IN ENZYMOLOGY

AND RELATED AREAS OF MOLECULAR BIOLOGY

Founded by F. F. NORD

Edited by ALTON MEISTER

CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, NEW YORK

VOLUME 50

1979

AN INTERSCIENCE ® PUBLICATION

JOHN WILEY & SONS
New York • Chichester • Brisbane • Toronto

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AND RELATED AREAS OF
MOLECULAR BIOLOGY

Volume 50

CONTRIBUTORS TO VOLUME 50

PETER J. BECHTEL, *Iowa State University, Animal Science Department, Ames, Iowa* 50011.

E. A. BELL, *Department of Plant Sciences, London* SE24 9JF, *England.*

ROSCOE O. BRADY, *Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland* 20205.

GERALD M. CARLSON, *University of Wisconsin, Institute for Enzyme Research, Madison, Wisconsin* 53706.

PETER H. FISHMAN, *Developmental and Metabolic Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland* 20205.

HEINZ G. FLOSS, *Purdue University, West Lafayette, Indiana* 47907.

L. FOWDEN, *Rothamsted Experimental Station, Harpenden, Herts, England.*

DONALD J. GRAVES, *Iowa State University, Department of Biochemistry and Biophysics, Ames, Iowa* 50011.

GEORGE D. HEGEMAN, *Department of Biology, Indiana University, Bloomington, Indiana* 47401.

GEORGE L. KENYON, *Department of Pharmacological Chemistry, University of California, San Francisco, California* 94143.

P. J. LEA, *Rothamsted Experimental Station, Harpenden, Herts, England.*

DAVID E. METZLER, *Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa* 50011.

IRWIN A. ROSE, *Institute for Cancer Research, Philadelphia, Pennsylvania* 19111.

MING-DAW TSAI, *Purdue University, West Lafayette, Indiana* 47907.

JAMES B. WALKER, *Department of Biochemistry, William Marsh Rice University, Houston, Texas* 77001.

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Library of Congress Catalogue Card Number: 41-9213

ISBN 0-471-05309-0

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

PREFACE TO VOLUME 50

It seems appropriate to comment on the publication of the fiftieth volume in this series. The first volume of *Advances in Enzymology* appeared in 1941 under the editorship of Friedrich F. Nord, who transplanted its predecessor, *Ergebnisse der Enzymforschung* (first published in 1932 in Leipzig) to the United States. This series has flourished since its inception; it has been fortunate in obtaining outstanding chapters by eminent authors, and the volumes have been warmly received by its readers. The study of enzymes has indeed been a moving force in the growth of biological knowledge, and enzymology continues to serve crucially in a broad range of disciplines.

It is perhaps instructive and certainly interesting to review the contents of the earlier volumes of *Advances in Enzymology,* which are given at the beginning of this volume. Even a brief inspection of the titles and authors gives one some feeling about the history and extraordinary development of biochemistry. The authors have included many of the pioneers in biochemistry and enzymology, and the chapters cover a broad range of topics. With the tremendous increase in scientific activity in recent years and the accompanying burgeoning scientific literature, many scientists and students have depended on authoritative and informative reviews to achieve a coherent picture of a particular field. Advances in Enzymology has provided a number of comprehensive reviews that serve this purpose and also has endeavored to provide chapters by outstanding scientists that emphasize their own thinking and accomplishments and thus serve as models of scientific exploration.

I wish to express my thanks and appreciation to those who have contributed chapters. It is gratifying to find that *Advances in Enzymology* continues to be widely read throughout the world; indeed, more than half of the copies are distributed outside the United States and the authorship of the chapters continues to be international. I also wish to express my appreciation to the publisher for continued encouragement and advice.

ALTON MEISTER

New York, New York
April 1979

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ADVANCES IN ENZYMOLOGY
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Volume 50

TAUTOMERISM IN PYRIDOXAL PHOSPHATE AND IN ENZYMATIC CATALYSIS

By DAVID E. METZLER, *Ames, Iowa*

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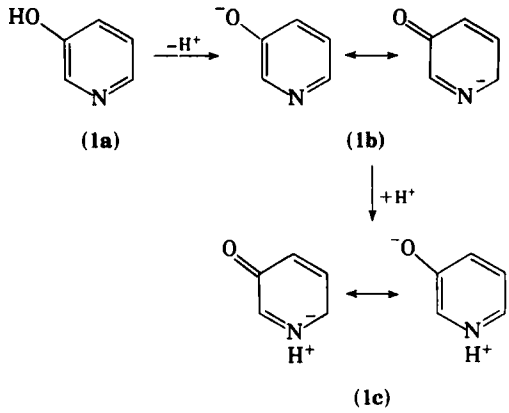
I. Introduction

The rapid equilibration of isomers in which one or more hydrogen atoms change positions is a prevalent phenomenon among biochemical substances. Although it has been suggested occasionally that such tautomerism is important in enzymatic catalysis, most enzymologists appear skeptical. The purpose of this chapter is to point out the magnitude of the electronic effects underlying tautomerism and to argue that tautomeric effects in enzymes probably play decisive roles both in catalysis and in regulation. A starting point is the coenzyme pyridoxal phosphate, but the chapter also deals with other coenzymes, with the peptide linkage and groups in amino acid side chains, and with purines and pyrimidines.

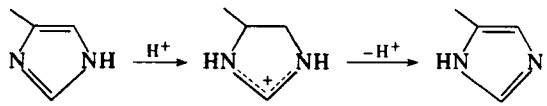
The underlying basis for tautomerism is the existence of a common resonance-stabilized structure formed by dissociation of a proton from any one of the set of tautomers. Let us consider 3-hydroxypyridine (1), whose anion **1b** (eq. 1) can be formed by dissociation of either of the tautomers **1a** and **1c**. As indicated in equation 1, the anion can be viewed as a hybrid of several resonance forms, some with the negative charge on oxygen and some with the charge in the ring. As a consequence, the

anion is able to accept a proton on either oxygen or nitrogen to give both tautomers **1a** and **1c**. At 25°C in aqueous solution the two are present in almost equal amounts (1,2).



(1)

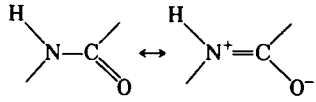
As indicated by the two resonance structures of the dipolar ion **1c**, the electrons of the anionic center are delocalized into the ring. This effect is so strong that the microscopic pK of the pyridinium ring proton is raised from the value of 5.4 observed for the cationic form of the compound to 8.3 in **1c**. An important consequence of this difference in pK is that protonation of **1c** on the oxygen at neutral pH causes release of the proton on the nitrogen, that is, **1c** is tautomerized to **1a**. The same property is shared by many other conjugated systems. For example, protonation of the free nitrogen of an imidazole ring leads to tautomerization (3). Such tautomerization, presumably occurring by synchronous addition of a proton on one nitrogen and removal from the other, provides the basis for the functioning of histidine in the "charge-relay" system of serine proteases. A similar process in which an iron ion replaces a proton may be important in the functioning of heme proteins.



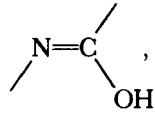
(2)

The amide linkage, present both in the peptide backbone and in side chains of proteins is a resonance hybrid of two forms (eq. 3). The amide group is very weakly basic with a pK value somewhat below 1. Both the

N and O atoms are of similar basicity (4) and a pair of tautomers, one most closely related to each of the structures in equation 3, can be formed by protonation on N or O.



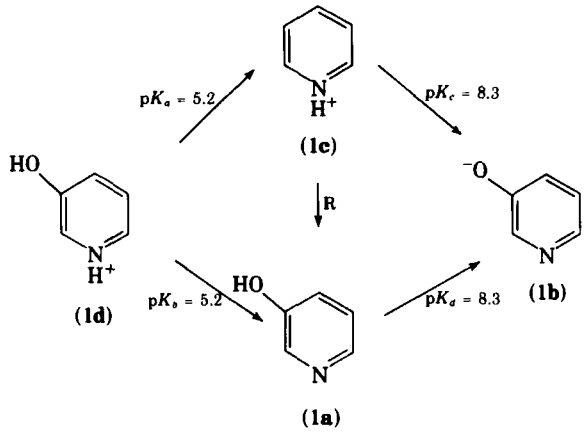
(3)

Furthermore, the possibility of tautomerization of the neutral amide group to an iminol form;  , in "high-energy" states of proteins should be considered. The amide groups of proteins associate in specific ways to make up hydrogen-bonded backbone structures such as α helices and β sheets. The internal hydrogen-bonded network of proteins often includes polar groups of side chains as well. Protonation or deprotonation of a group at one end of a chain of such hydrogen-bonded groups is capable of inducing an electrostatic effect at a distance through the chain. It is argued in this chapter that such effects in all probability are basic to catalysis, as well as to control of enzymatic activity by allosteric and covalent modifications and to a variety of other biological phenomena.

II. Tautomerism of 3-Hydroxypyridines

Let us consider quantitatively 3-hydroxypyridine itself. Experimentally two stepwise dissociation constants can be determined by titration or by spectrophotometry. At 25°C in water these have values of about 4.9 and 8.6 (1,2). The microscopic dissociation constants corresponding to individual groups in the molecule may be designated K_a, K_b, K_c, and K_d as indicated in equation 4. These constants are related to the experimental quantities K_1 and K_2 and to the tautomeric ratio R, a pH-independent quantity that represents the equilibrium constant for tautomerization of **1c** to **1a**. In most instances of tautomerism it is difficult to determine the ratio R directly. It is customary to solve the problem in another way by using methylated derivatives to give an estimate of one of the four microscopic constants. If one of these constants is known, the other three can be determined from K_1 and K_2 . In the case of 3-hydroxypyridine, R can be measured directly by observation of the

absorption spectrum since **1c** absorbs at 314 nm and **1a** absorbs at 275 nm. By changing the polarity of the solvent, for example, by using water–methanol mixtures, it is possible to change R from 1.0 in water to about 33 in 80% methanol and to a much higher value in pure methanol. By observing changes in the areas under each of the two absorption bands caused by a change in solvent, the relative molar areas (integrated intensities) of the two bands and a precise value of R may be determined (2). Basic to this method is the use of log normal distribution curves that fit absorption bands of aromatic molecules exceedingly well and permit accurate determination of areas of overlapping bands (2,5–7).



(4)

The four microscopic constants in aqueous solution are indicated in equation 4. The magnitude of the electrostatic interaction between the two groups on the ring is illustrated by comparing K_a and K_d, and K_b and K_c. The change of three units in the pK for the same group as a result of protonation or deprotonation of the other group amounts to an energy of interaction of 17 kJ/mol. The effect is to allow the two functional groups in **1d** to have nearly the same basicity.

Tautomerism also occurs in substituted 3-hydroxypyridines. For example, as shown in Table I, pyridoxine (**2**) has pK values very similar to those of 3-hydroxypyridine, but the slightly larger spread between pK_a and pK_d causes the tautomerization constant R to decrease. About three quarters of the molecules exist as the dipolar ionic tautomer. Notice that the equilibrium is dependent on temperature as well as on solvent (Table I). The coenzyme pyridoxamine phosphate (**3**) likewise undergoes tautomerism but of a more complex sort because of the participation of