NUCLEOSIDES AS BIOLOGICAL PROBES

Robert J. Suhadolnik

NUCLEOSIDES AS BIOLOGICAL PROBES

ROBERT J. SUHADOLNIK

Department of Biochemistry
Temple University
School of Medicine

A WILEY-INTERSCIENCE PUBLICATION

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

Library of Congress Cataloging in Publication Data

Suhadolnik, Robert J 1925–
Nucleosides as biological probes.

"A Wiley-Interscience publication."
Includes index.
1. Nucleosides—Physiological effect.
2. Nucleosides—Metabolism. I. Title.

QP625.N88S93 574.8'76'028 79-10719
ISBN 0-471-05317-1

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Preface

In this book a detailed, up-to-date emphasis is placed on the application of the 70 known naturally occurring nucleoside analogs as biochemical probes in cellular reactions. Nine years ago, when my book, *Nucleoside Antibiotics*, was published, our knowledge of the nucleoside analogs as biochemical probes was not as clearly defined. Much unexpected information has been obtained concerning the simple and complex enzyme reactions, macromolecular syntheses, viral replication, and cell wall formation. This vast accumulation of knowledge has made it possible to complete the difficult transition of taking nucleoside analogs from the laboratory to their use in the treatment of human diseases.

Many interesting and exciting developments have been revealed during these last 9 years in terms of unique structures and physical, chemical, biosynthetic, pharmacological, and biochemical properties. Nine years ago the 30 known naturally occurring nucleoside analogs were diverse both in their structure and their activity against animals, plants, bacteria, and viruses. They had found valuable use in the elucidation of the complex steps involved in reading the genetic message on the ribosomes for protein, RNA and DNA synthesis and in the regulation of purine and pyrimidine nucleotide biosynthesis, enzyme reactions, chitin synthesis, and subcellular organization. Many of the 33 newly discovered analogs have found continued use in the above cellular processes, but also have expanded properties such that they are not only antagonists of purine and pyrimidine nucleotides but exert other physiological actions on humans.

The recently discovered analog tunicamycin, which is a structural analog of UDP-*N*-acetylglucosamine and dolichol, has been especially useful in studies related to glycoprotein and peptidoglycan synthesis in animals, plants, bacteria, and viruses. Similarly, puromycin, which has been so valuable as a codon-independent functional analog of aminoacyl-tRNA is now used as the *p*-azido photoaffinity label to form covalent bonds with the ribosomal proteins to more clearly define the exact protein with which puromycin reacts.

Cordycepin, which was originally reported to be a false feedback inhibitor of purine nucleotide biosynthesis as well as RNA synthesis, has been of great value as a biochemical probe in the synthesis of poly(A) and eventually the formation of functional, cytoplasmic mRNA. Three of the newly discovered analogs are extremely useful immunosuppressive agents (coformycin, 2′-deoxycoformycin, and bredinin). Coformycin and 2′-deoxycoformycin are also potent inhibitors of adenosine deminase. As such, they prevent normal mammalian and tumor cells from deaminating adenosine analogs (i.e., cordycepin, formycin, and ara-A). This allows for a marked increase in the concentration of the 5′-triphosphate of the analogs such that the tumor cell is bathed in much higher concentrations of the analogs for prolonged periods of time. The net result is a marked increase in the therapeutic efficiency of the drug. A new concept of inhibition by adenosine analogs via a "nucleotide independent" mechanism has been recently introduced. Ara-A and cordycepin bind irreversibly by first order kinetics to S-adenosylhomocysteine hydrolase, which is a "target" enzyme for these adenosine analogs. The result is a "suicide" inactivation (see Chapter 4).

Eritadenine, an adenine analog, is not a true nucleoside analog. However, it is reviewed here because it has the property of decreasing plasma cholesterol. Clitidine, one of two pyridine nucleoside analogs, has powerful hypoesthetic and hyperemic activity in mammals. Two new analogs, thuringiensin and agrocin 84, are elaborated as the nucleotides and exert their inhibitory activity as the nucleotides. Since 1970, new data have been accumulated showing that crotonoside and showdomycin can distinguish the inducible constitutive binding sites on the bacterial cell wall. Decoyinine, the ketohexose purine analog, is used to induce sporulation. One of the new analogs of guanosine, 2′-amino-2′-deoxyguanosine, has also been discovered. Bredinin, a nucleoside analog of AICA, in addition to its immunosuppressive properties and inhibitor of RNA and protein synthesis, has potent antiarthritic antirheumatoid activity.

Another important finding since 1970 is the report of the pyrrolopyrimidine ring in Q base of tRNA of mammalian tissue. Prior to this, the pyrrolopyrimidine aglycon was found in the nucleoside analogs, tubercidin, toyocamycin, and sangivamycin. The biosynthesis in the eukaryote and the prokaryote uses guanine as the carbon–nitrogen skeleton.

Finally, in 1970 only 5-azacytidine had found limited use in the treatment of human leukemia. None of the nucleoside analogs had FDA clearance. Today, tubercidin and ara-A have been cleared by the FDA for the treatment of human basal cell carcinoma, herpes simplex keratitis, and herpes simplex encephalitis. In terms of structures, the carbohydrate moiety of the octosyl acids and the ezomycin complex is a new type trans-fused anhydrooctouronic acid.

This volume on the nucleosides as biological probes is structured on the basis of the biological role that most accurately describes each nucleoside/nucleotide analog in cellular processes. Emphasis is also placed on the pleotropic effect of each analog to assist the reader in the multifunction activity of the analogs.

For uniformity, the newly discovered nucleoside analogs are presented according to the following plan: introductory comments; Discovery, Isolation, and Production; Physical and Chemical Properties; Structural Elucidation; Chemical Synthesis; Synthesis of Analogs; Inhibition of Growth; Biosynthesis; Biochemical Properties; Summary; and References. Sections concerning those nucleoside analogs reviewed in *Nucleoside Antibiotics* (Wiley, 1970) contain only the Synthesis; Biosynthesis; Biochemical Properties; Summary; and References. The reader is referred to this edition for supplemental reading.

Finally, I have carefully read and reviewed more than 4000 publications related to the naturally occurring nucleoside/nucleotide analogs presented in this revised edition. The preparation of this book was made possible by the numerous reprints, preprints, and personal detailed data made available to me by the many scientists throughout the world who have been actively engaged in research with these nucleosides and those who have given generously of their valuable time in editing the chapters sent to them.

ROBERT J. SUHADOLNIK

Philadelphia, Pennsylvania
June 1979

Acknowledgments

I should especially like to thank the following colleagues for their valuable editorial comments, suggestions, and criticisms: Drs. R. Agarwal, A. Argoudelis, O. Bârzu, L. Bennett, Jr., G. Bettinger, A. Bloch, V. Bohr, R. Borchardt, B. Brdar, A. Bruzel, C. Cheung, S. Cohen, B. Cooperman, J. Coward, F. Cramer, J. Darnell, E. De Clercq, P. Doetsch, J. Doskočil, J. Drach, K. Eckardt, A. Elbein, N. Fedorinchik, J. Fox, M. Franze-deFernandez, E. Freese, G. Gentry, A. Glazko, G. Gentry, G. Gutowski, H. Hadler, R. Hamill, R. Hamilton, L. Hanka, T. Haskell, A. Holý, M. Hori, J. Horwitz, L. Hurley, M. Ikehara, K. Isono, K. Iwai, D. Johnson, G. Just, T. Kalman, A. Kerr, T. Kishi, W. Klassen, H. Kneifel, W. König, J. Lampen, L. Lerner, W. Lennarz, F. Lichtenthaler, D. Lichtenwalner, C. Lum, R. Majima-Tsutsumi, J. Moffatt, K. Moldave, S. Morimoto, J. Mosca, W. Müller, R. Nagarajan, S. Nakamura, T. Nakanishi, J. Nevins, R. Parks, S. Pestka, H. Pitot, W. Plunkett, C. Pootjes, M. Robins, R. Robins, K. Sakaguchi, T. Sakagami, K. Sakata, K. Šebesta, J. Schell, V. Shchepetil'nikova, H. Seto, C. Smith, F. Šorm, A. Takatsuki, J. Tkacz, S. Tokuda, I. Townsend, J. Veselý, R. Vince, D. Visser, K. Watanabe, M. Weigele, R. Whitley, J. Wu, I. Zaenen, H. Zähner, J. Žemlička.

I also wish to express my sincerest appreciation to Nancy L. Reichenbach for her valuable assistance and patience in the assembling and organization of the chapters, tables, permissions, drawing of the structures of the analogs, and many of the figures. Without these many hours of dedication, the final publication would have been greatly delayed.

R.J.S.

Contents

Chapter 1 Inhibition of Cell Wall Synthesis, Cell Wall Receptors and Transport, Viral Coat Formation, Fungi, and Yeast

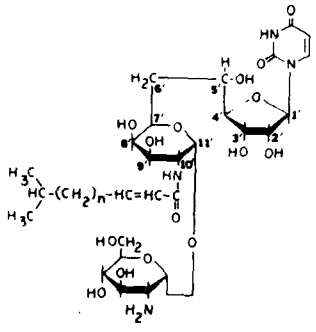
---

This chapter reviews the eukaryotes, prokaryotes, and viruses as they are affected by the naturally occurring nucleoside/nucleotide analogs that inhibit (i) cell wall synthesis (i.e., tunicamycin and the streptovirudins), (ii) fungi (i.e., sinefungin, the ezomycins, the polyoxins, thraustomycin, amipurimycin, nikkomycin, and septacidin), (iii) yeast (i.e., platenocidin, and sinefungin) and (iv) cell wall receptors and transport (i.e., agrocin 84,

showdomycin, and crotonoside). The polyoxins, showdomycin, crotonoside, and septacidin, have been reviewed in detail (Suhadolnik, 1970) and are discussed here only in terms of studies related to their biological properties since 1970. The remaining nucleoside analogs are reviewed in detail with respect to discovery, inhibition of growth, chemical, physical, and biochemical properties, and biosynthesis.

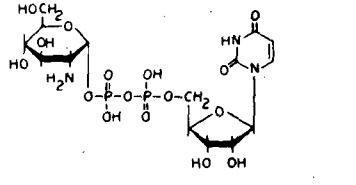
1.1 TUNICAMYCIN AND THE STREPTOVIRUDINS

Some 60–70 naturally occurring nucleoside analogs have been widely used as biochemical probes for many complex cellular reactions. An equally valuable analog is tunicamycin (TM) (Fig. 1.1), discovered by Takatsuki et al. (1971), which is a structural analog of UDP-*N*-acetylglucosamine and dolichol. Takatsuki and Tamura (1971a, b) were the first to demonstrate that TM selectively inhibits the incorporation of sugars into acid-insoluble compounds and the first to show that TM inhibits the multiplication of Newcastle disease virus. Takatsuki and coworkers were also the first to report that TM induces morphological changes in bacteria and yeast (Takatsuki et al., 1971, 1972). TM prevents glycosylation of proteins by specifically inhibiting the formation of the lipid-linked sugar intermediate. TM was first isolated by Takatsuki et al. (1971) from the mycelium and the culture filtrates of *Streptomyces lysosuperficus*. Ito et al. (1977) and Takatsuki et al. (1977a) have shown that TM is not a single compound but, rather, a mixture of homologous antibiotics. There appear to be at least



TUNICAMYCIN

TUNICAMYCIN A n=9
TUNICAMYCIN B n=10
TUNICAMYCIN C n=8
TUNICAMYCIN D n=11



UDP-N-ACETYL-α-D-GLUCOSAMINE

Figure 1.1 Structure of the tunicamycin complex and comparison to UDP-*N*-acetyl-α-D-glucosamine.

tunicamycins A, B, C, and D (Fig. 1.1). Based on the elegant degradations and physical data obtained by Ito et al. (1977), the structure has been assigned for the main components of the TM complex. Each contains uracil, a fatty acid component that is a trans α,β-unsaturated branched chain acid, and *N*-acetylglucosamine. Although TM and the polyoxins (see page 30) are structural analogs of UDP-*N*-acetylglucosamine, the polyoxins, without the unsaturated branched chain acid, are limited to the competitive inhibition of chitin synthetase. The structure of TM has two features, that is, the nucleoside part of TM is an analog of the nucleoside diphosphate sugars, and the unsaturated acid part is an analog of dolichol. It is these structural features of TM that make it an analog of dolichyl-*N*-acetyl-glucosaminyl pyrophosphate which inhibits the transfer of *N*-acetylglucosamine from the lipid saccharide required for the assembly of the oligosaccharide component of glycoproteins and peptidoglycan. A new C_{11} aminodeoxydialdose, named tunicamine, has also been isolated and characterized (Ito et al., 1978). Hydrolysis of TM with 3 *N* HCl, 3 h, at 100°C cleaves TM to tunicaminyl uracil, D-glucosamine, and several unsaturated fatty acids. Isopentadecanoic acid is present in addition to the fatty acid already mentioned (Takatsuki et al., 1977a). TM, which derives its name from the latin, *tunica*, meaning coat, inhibits gram-positive bacteria (especially *Bacillus* species), yeast, fungi, protozoa, plants, envelope viruses, and mammalian cells in culture (Takatsuki et al., 1971). Because of its ability to inhibit the synthesis of *N*-acetylglucosaminyl-lipids in prokaryotes, and eukaryotes, tunicamycin is one of the most useful biochemical probes for elucidation of the complex reactions involved in the assembly of glycoproteins and cell walls. Tunicamycin differs from other glycolipid antibiotics such as diumycin, macarbomycin, moenomycin, and prasinomycin in that it does not contain phosphorus; however, TM is capable of inhibiting the transferases involved in glycolipid synthesis in prokaryotes, eukaryotes, and viral coat formation. Although the streptovirudins differ chemically from TM, their primary action also involves the inhibition of glycoprotein synthesis (J. S. Tkacz, personal communication). The antibiotic mycospocidin, which was isolated by Nakamura et al. (1957), is in the same class as the TM and streptovirudin groups (Eckardt et al., 1973; K. Eckardt, personal communication; J. Tkacz, personal communication). For reviews, see Lambert et al. (1977), Kuo and Lampen (1972), and Waechter and Lennarz (1976).

Discovery, Production, and Isolation

Takatsuki et al. (1971) were the first to report on the isolation and production of TM from *S. lysosuperficus*. TM is isolated from either the cells or

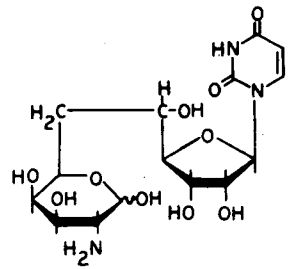
the culture filtrates. The medium, growth conditions, and isolation of TM have been described in detail (Takatsuki et al., 1971).

Physical and Chemical Properties

Tunicamycin is a white crystalline powder; mol wt ~ 870; mp 234–235°C; $[\alpha]_D^{20}$ +52° (*c* 0.5, pyridine); ultraviolet spectral properties: $\lambda_{max}^{methanol}$ 250 nm ($E_{1cm}^{1\,percent}$ 230), 260 nm ($E_{1cm}^{1\,percent}$ 110); the nmr spectrum of TM has been reported; treatment with periodate results in a rapid loss of antiviral activity (Takatsuki et al., 1971).

Structural Elucidation

Takatsuki et al. (1977a) and Ito et al. (1978) have reported on the structure of some members of the TM complex (Fig. 1.1). Hydrolysis of TM in 3 *N* HCl, 100°C, 3 h results in the isolation of four major *trans*-α,β-unsaturated *iso* acids (Takatsuki et al., 1979; Ito et al., 1978) and tunicaminyl uracil, 1-β-D-(10′-amino-6′,10′-dideoxy-L-galacto-D-allo-undecadialdo-7′,11′-pyranose-1′,4′-furanosyl)uracil. The ultraviolet spectral properties are as follows: $\lambda_{max}^{H_2O}$ 261 nm (ϵ = 10,700); mass spectra and nmr have been described (Takatsuki et al., 1977a). Ito et al. (1978) have proposed the name tunicamine for the dialdose with 11 carbons attached to uracil. Crystalline D-



TUNICAMINYL URACIL

glucosamine is isolated from the hydrolyzed TM and the α,α-linkage between C-11 of tunicamine and C-1″ of *N*-acetylglucosamine has been assigned on the basis of the high molecular rotation of TM and their spectrum; the permethylated derivative of TM complex showed peaks at *m/e* 970, 984, 998, and 1012, which might be assigned to M^+ ion peaks of permethylated tunicamycins C, A, B, and D, respectively (Takatsuki et al., 1977a). The major structural differences between the streptovirudin complex

(series II) and the tunicamycin complex are in their fatty acid chains (J. S. Tkacz, personal communication; Eckardt et al., 1975).

Inhibition of Growth

Tunicamycin is active against plant and animal RNA and DNA viruses, gram-positive bacteria, yeast, and fungi (Takatsuki et al., 1971; Takatsuki and Tamura, 1971a). *Bacillus* species are the most sensitive bacteria studied. Tunicamycin induces various morphological changes among the organisms sensitive to the antibiotic without inhibiting protein, RNA, or DNA synthesis.

Biochemical Properties

Effect on Eukaryotes. Tunicamycin has been used as a biological probe to study the assembly, secretion and function of glycoproteins in mammalian cells, bacteria and viruses (Lucas et al., 1975; Chen and Lennarz, 1976). Elbein and coworkers (Heifetz et al., *Biochemistry*, **18,** 2186, 1979; ref. added in pg. proof) have reported that TM is a noncompetitive inhibitor of GlcNAc 1-phosphate transferase (Fig. 1.2), inhibits the reverse reaction and is a substrate-product transition-state analog. The synthesis of the polypeptide chain of ovalbumin has been reported in great detail by Shimke and coworkers (Palmiter et al., 1971). Keiley et al. (1976) documented that nascent chains of ovalbumin are still attached to tRNA when the carbohydrate chain of mannose and *N*-acetylglucosamine units are added.

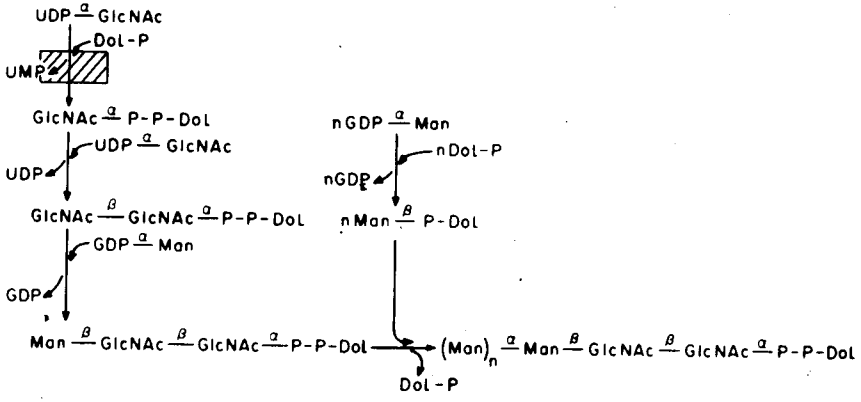


Figure 1.2 Lipid-linked sugars in glycoprotein synthesis. Postulated sequence in assembly of the oligosaccharide-lipid based on preparations of liver, myeloma, pancreas, lymphocyte, and oviduct membrane. The known site of inhibition by tunicamycin is shown by the crosshatched bar. Modified from Waechter and Lennarz, 1976.