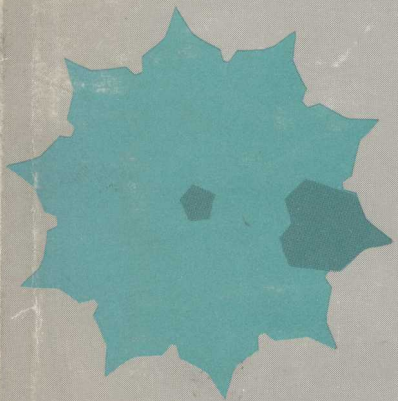
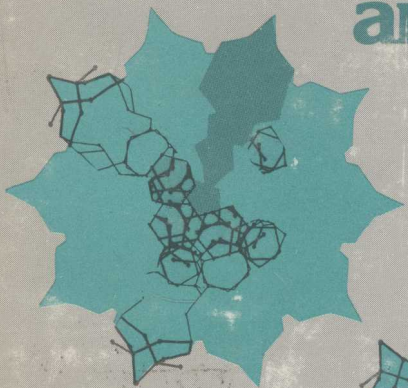
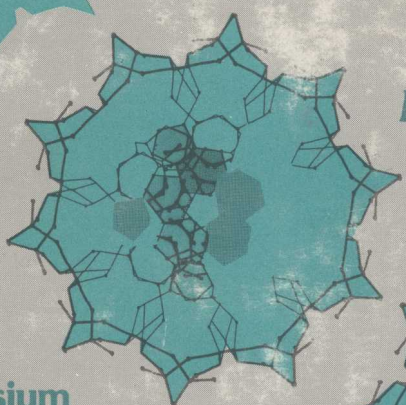
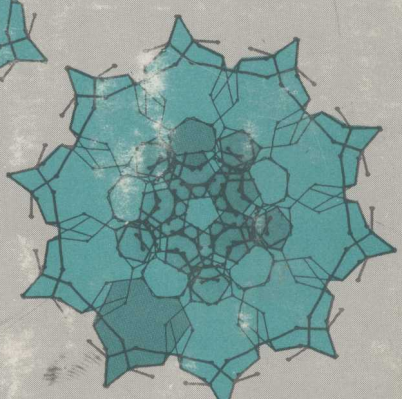
Conformationally Directed Drug Design

Peptides and Nucleic Acids as Templates or Targets









EDITED BY
Julius A. Vida
Maxwell Gordon

ACS Symposium Series
251

ACS SYMPOSIUM SERIES 251

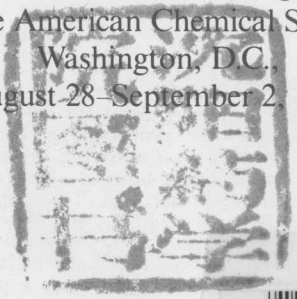
Conformationally Directed Drug Design

Peptides and Nucleic Acids as Templates or Targets

Julius A. Vida, EDITOR
Bristol-Myers Company

Maxwell Gordon, EDITOR
Bristol-Myers Company

Based on a symposium sponsored by
the Division of Medicinal Chemistry
at the 186th Meeting
of the American Chemical Society,
Washington, D.C.,
August 28–September 2, 1983



American Chemical Society, Washington, D.C. 1984

Library of Congress Cataloging in Publication Data

Conformationally directed drug design.
(ACS symposium series, ISSN 0097-6156; 251)

Includes bibliographies and indexes.

1. Chemistry, Pharmaceutical—Congresses.
2. Peptides—Congresses. 3. Nucleic acids—Congresses.
4. Structure-activity relationship (Pharmacology)—
Congresses. 5. Stereochemistry—Congresses.

I. Vida, Julius A., 1928– . II. Gordon, Maxwell,
1921– . III. American Chemical Society. Division of
Medicinal Chemistry. IV. American Chemical Society.
Meeting (186th: 1983: Washington, D.C.) V. Series.
[DNLM: 1. Nucleic acid conformation—Congresses.
2. Protein conformation—Congresses. 3. Chemistry,
Pharmaceutical—Congresses. QU 58 C7487 1983]

RS401.C66 1984 615'.31 84-2921
ISBN 0-8412-0836-0

PRINTED IN THE UNITED STATES OF AMERICA

Conformationally Directed Drug Design

ACS Symposium Series

M. Joan Comstock, *Series Editor*

Advisory Board

FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

PREFACE

TRADITIONAL MEDICINAL CHEMICAL RESEARCH has involved the testing of drugs on whole animals or isolated organ systems for a desired end result. The drugs themselves were usually arrived at empirically through screening, or they represented analogs of compounds previously found to show the desired activity.

In the new era of medicinal chemistry, drug design is based on the structure of effector molecules or receptor sites. Thus, cimetidine was designed to be a specific histamine antagonist, captopril was designed to inhibit the angiotensin-converting enzyme, and so forth. Using powerful new developments in immunology, molecular biology, computer modeling, and recombinant techniques, scientists can study receptor sites from the point of view of three-dimensional structure and can design antagonists specific to the action of drugs or transmitter substances at those sites. In particular, the conformation of biopolymers and their tertiary structures is amenable to X-ray analysis or other powerful techniques for determining structure.

Many studies report the use of peptide and nucleic acid conformation as a tool for drug design. Analogs of somatostatin having greater potency and stability than the prototype have been designed from a model of the prototype. In one study the design of an affinity label for creatine kinase demonstrated how such information could be used in the search for agents directed at an enzyme's active site.

One series of investigations has shown that a rational, conformationally based approach to analog design requires supplementary information, over and above simple sequence data. One tool was the introduction of highly constrained transannual bridges to construct specific antagonists of active peptides. In other studies free synthetic peptides have proven to be powerful reagents for inducing specific tolerance to preselected regions of a protein and for preparing antibodies having specificities for preselected protein regions.

In this volume many investigators report on their own studies in conformationally directed drug design. The common theme of all the chapters is the importance of the conformational structure of peptide and nucleic acid in the design of drugs that are either peptide- or nucleic-acid-based or that interact with peptides or nucleic acids. We anticipate that at the present rate of progress significant therapeutic advances will result from

these investigators' efforts. We are grateful to the authors for their contributions and for the privilege of collaborating with them on the publication of this volume.

JULIUS A. VIDA
MAXWELL GORDON
Bristol–Myers Company
New York, New York

January 1984

CONTENTS

Virus–Receptor Interactions

BERNARD N. FIELDS

Department of Microbiology and Molecular Genetics, Harvard Medical School, and Department of Medicine (Infectious Disease), Brigham and Women's Hospital, Boston, MA 02115

MARK I. GREENE

Department of Pathology, Harvard Medical School, Boston, MA 02115

In this discussion we have considered certain protein-protein interactions determined by the binding of reovirus hemagglutinin with cell receptors. A specific epitope on the reovirus hemagglutinin identified by the G5 monoclonal antibody is critical for binding reovirus type 3 to cell surface receptors. Monoclonal anti-idiotypic reagents have been generated which resemble the virus in terms of its ability to interact with cell receptors. The monoclonal anti-idiotype interacts with the monoclonal antibodies of the G5 neutralization epitope specificity. This idiotype-anti-idiotype system mimics the protein-protein interaction observed with binding of the virus hemagglutinin to cell surface receptor. Hence anti-idiotypes are anti-receptor. Such anti-receptor antibodies might also be used in the development of vaccines for certain pathogenic organisms since the anti-idiotypic antibodies resemble the ligand.

The mammalian reoviruses are segmented double-stranded RNA viruses (1). Of the various proteins of reovirus, the hemagglutinin appears to play a predominant role in determining tropism of the virus for different cell tissues. This has been appreciated as a consequence for the genetic analysis of the reovirus serotypes (2). For example, reovirus type 3 has a tropism for neuronal cells but spares ependymal cells. Reovirus type 1, on the other hand, has a tropism for ependymal cells and spares neuronal cells damage (3). Recombinant viruses have been developed which have all the genes of type 3 except for the gene encoding the hemagglutinin of type 1, (this recombinant is termed 3.HA1). Similarly, viruses have been developed which have all the

0097-6156/84/0251-0001$06.00/0

genes for type 1 except the gene encoding the hemagglutinin of type 3 (this recombinant is termed 1. HA3). Use of these viral recombinants has shown that the viral hemagglutinin specifies tropism for the neuronal and ependymal cells discussed above.

The hemagglutinin is also a major immunogen in terms of the immune response (4). The greater portion of the cytolytic T cell response is directed at the reovirus hemagglutinin in association with histocompatibility proteins (5). Thus animals immunized with reovirus type 3 generate cytolytic T cells which have the capacity to lyse and infect histocompatibility matched reovirus type 3 infected targets but not a reovirus type 1 infected target. Similarly, 1.HA3 infected targets are lysed whereas 3.HA1 infected targets are not lysed appreciably. The targets can be protected from lysis with antibodies directed to the hemagglutinin or by anti-H-2 antibodies (6).

Antibody response; structural characteristics

At the level of the B cell the antibody response to the hemagglutinin has been studied in great detail. The response is markedly oligoclonal with a cluster of HA specific immunoglobulins with a PI of between 6.9 and 7.1 (7). The antibody response to the hemagglutinin of type 3 is also typified by the presence of a structural determinant known as a shared or cross-reactive idiotypic determinant (Figure 1) expressed in many of the antibodies with specificity for the type 3 hemagglutinin. The idiotype has been identified by rabbit antibodies direct at anti-hemagglutinin immunoglobulins. The anti-idiotype-idiotype interaction is inhibitable by free hemagglutinin. Hence the idiotype (see below) is associated with the antigen conbining site of the immunoglobulin protein. Hence in the response to hemagglutinin a homogeneous structurally similar antibody response is induced (8).

Hemagglutinin topography

A panel of monoclonal antibodies has been developed and used to analyze various regions of the hemagglutinin (9,10). Three distinct epitopes have been defined for the reovirus type 3 hemagglutinin. (Figure 2) One region is important for neutralization. Similarly, distinct monoclonal antibodies determine the spatially separate hemagglutinin inhibition epitope, and other antibodies distinguish a third region which has not yet been functionally defined. Of interest in the screening of these monoclonal antibodies was the fact that one of them, termed G5, expresses similar cross-reactive idiotypic determinants as antibodies with hemagglutinin

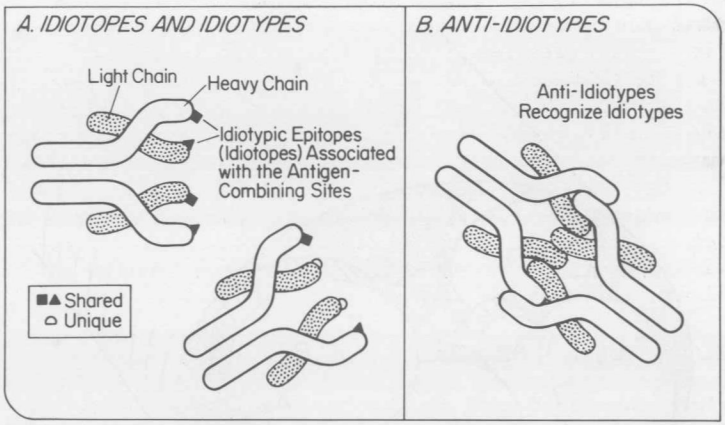


Figure 1. A general scheme of idiotypes and anti-idiotypes. Depicted here are immunoglobulin proteins whose antigen-combining site expresses unique conformations or epitopes, that are termed idiotopes. A collection of idiotopes constitute what is termed idiotype. Some idiotypes are shared by many immunoglobulins with the same antigenic specificity.

Anti-idiotypes are antibodies with specificity for idiotypes. The antibodies can be made in the same species or in other species. Often the interaction of anti-idiotypes to idiotypes is inhibited by free antigen.

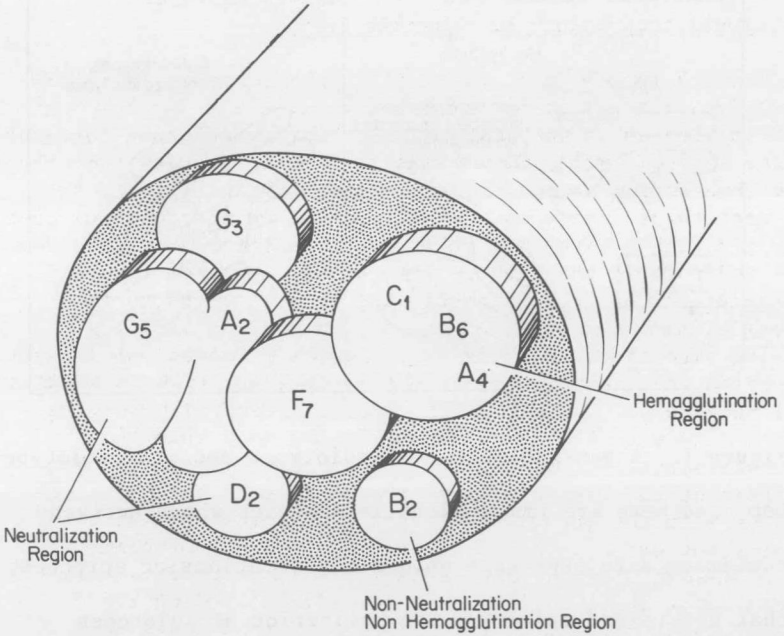


Figure 2. Topography of the reovirus hemagglutinin. Depicted here in this cartoon is a representation of various domains of the reovirus hemagglutinin. Some domains are overlapping whereas others are separate and unique from all the rest. The G5 domain appears to be the most relevant to the immune response and also for tropism.

specificity (9). Studies focusing on the G5 region of the hemagglutinin protein have indicated that this epitope is important in the tropism of the virus. Immunoselection of variant reoviruses by using G5 antibody has shown that viruses that resist neutralization by G5 are attenuated and show altered tropism (11).

Monoclonal anti-idiotypes resemble ligand

The network theory of N. Jerne (12) proposes that antibodies themselves can act as antigens and provoke the synthesis of anti-antibodies in the host animal. The immunogenic portions of the antibody molecule as suggested above results from the structure in and around the antigen combining site and are referred to as idiotypes. As discussed, immunoglobulins that recognize these sites are referred to as anti-idiotypes. We reasoned that if the binding site of the reovirus type 3 hemagglutinin on cell receptors and on antibodies to the hemagglutinin were similar, antibodies made against the binding site of one protein (such as the G5 monoclonal) might recognize the binding site of other proteins (such as somatic cell receptors). To test this idea, by repeatedly priming BALB/c mice with G5 monoclonal cells, we were able to generate syngeneic monoclonal anti-idiotype reagents. The analysis of the interaction of the monoclonal anti-idiotype binding with the monoclonal G5 antibody revealed that this interaction was determined through the antigen-combining site. When monoclonal anti-hemagglutinin antibodies were assessed for their ability to bind radiolabeled purified hemagglutinin protein in the presence of monoclonal-anti-idiotype, there was marked inhibition (8). Thus, the monoclonal anti-idiotype and hemagglutinin bind to the same region of the antigen-combining site of G5 antibodies. This indicates that the monoclonal anti-idiotype functionally resembles the hemagglutinin neutralization domain epitope. We have analyzed further the protein-protein interactions which are important in viral receptor binding by a variety of approaches. Many of these approaches have in common the use of monoclonal anti-idiotype. For example, it has been possible to show that reovirus type 3 binds to a subset of murine cells or to a panel of lymphoid lines that have been maintained *in vitro*. Exposure of monoclonal anti-idiotype to the cell lines at the time of exposure to mammalian reovirus blocks the binding of reovirus type 3 (13). Monoclonal anti-idiotype is thus capable of interfering with viral interaction with the mammalian reovirus receptor on these cell lines. These observations suggest that monoclonal anti-receptor antibodies might represent a new approach for preventing virus binding, an event necessary for infection.

Monoclonal anti-idiotypic reagents were also assessed for their ability to prime for cellular immunity to mammalian reoviruses. We reasoned that if the anti-idiotype resembled the viral hemagglutinin enough to recognize the surface receptors, it might stimulate antibodies that in turn recognize the viral hemagglutinin. Such a result would indicate that anti-idiotype could serve as a potential vaccine (4). In order to evaluate whether the monoclonal anti-idiotypic reagent could be used as a vaccine in a syngeneic system, BALB/c mice were immunized with 200 microliters of clarified ascites fluid derived from animals bearing the anti-idiotypic hybridoma. Animals were challenged in the footpad 5 days later with virus. Animals primed with anti-idiotype developed a T cell-dependent inflammatory response in vivo to mammalian reovirus type 3, type 1.HA3, and not to type 1 or type 3 HA1. Thus these results suggest that monoclonal anti-idiotypes resemble the viral hemagglutinin biologically and might be of use in the immunization against certain pathogenic agents. (Figure 3)

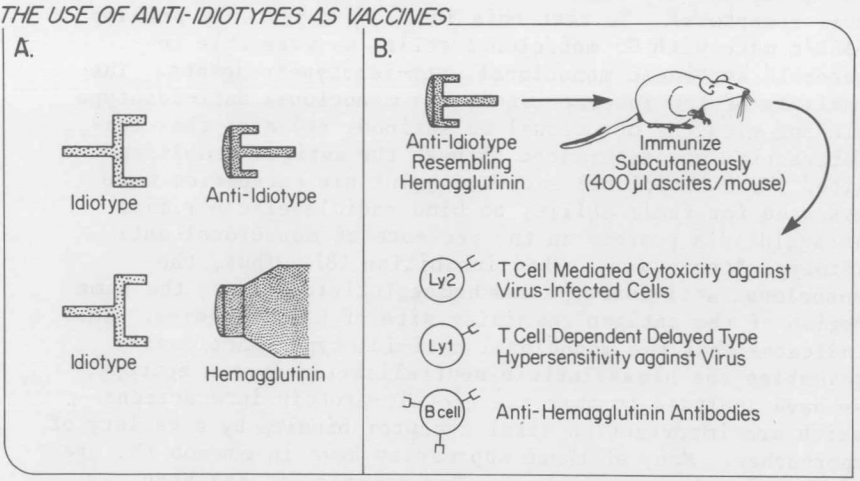


Figure 3. The use of anti-idiotypes as vaccines. The anti-idiotypes in the reovirus system resemble hemagglutinin neutralization domains. Functionally shown here is the consequence of administering monoclonal anti-idiotypic proteins to mice. A variety of reovirus specific immune reactivities are induced.

Literature Cited

1. Joklik W.K. Reproduction of reoviridal. In "Comprehensive Virology" (H. Fraenkel-Conrat and R. Wagner, eds.) 1974, New York: Plenum Press, Vol. 2, pp 231-334.
2. Fields B.N. 1982. Arch. Virol. 71:95-107
3. Weiner H.L., Drayna D., Averill D.R. Jr. and Fields B.N. 1977. Proc. Natl. Acad. Sci. U.S.A. 74:5744.
4. Fields B.N. and Greene M.I. Nature 1982 300:19.
5. Finberg R., Weiner H.L., Fields B.N., Benacerraf B. and Burakoff S.J. 1979 Proc. Natl. Acad. Sci. U.S.A. 76:442.
6. Finberg R, Spriggs D.R. and Fields B.N. 1982. J. Immunol. 129:2235.
7. Nepom J.T., Weiner H.L., Dichter M.A., Spriggs D., Gramm C.F., Powers M.L., Fields B.N. and Greene M.I. 1982 .J. Exp. Med 155:155.
8. Noseworthy J.H., Fields B.N., Dichter M.A., Sobotka, C., Pizer E., Perry L.L., Nepom, J.T. and Greene M.I.J. Immunol. 1983, in press.
9. Burstin S.J., Spriggs D.R. and Fields B.N. 1982 Virology 117:146.
10. Spriggs D.R., Kaye K. and Fields B.N. 1983 Virology 12:71.
11. Spriggs D.R. and Fields B.N. 1982 Nature 297:68.
12. Jerne N.K. 1974 Ann. Immunol. (Inst. Pasteur) 125c:373.
13. Unpublished data.

RECEIVED November 15, 1983