Molecular Architecture of Proteins and Enzymes

Edited by

Ralph A. Bradshaw

Department of Biological Chemistry
California College of Medicine
University of California, Irvine
Irvine, California

Jordan Tang

Laboratory of Protein Studies
Oklahoma Medical Research Foundation
University of Oklahoma, Health Science Center
Oklahoma City, Oklahoma

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Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

David E. Ash (31), *Department of Biochemistry, Temple University School of Medicine, Philadelphia, Pennsylvania 19122*

Charles E. Bugg (65), *Departments of Biochemistry and Microbiology, Comprehensive Cancer Center and Institute of Dental Research, University of Alabama in Birmingham, Birmingham, Alabama 35294*

Graham Carpenter (175), *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

T. Chandra (235), *Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

Chang Wen-rui (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Giovanna Colombo (31), *Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802*

Dai Jin-bi (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Steven E. Ealick (65), *Departments of Biochemistry and Microbiology, Comprehensive Cancer Center and Institute of Dental Research, University of Alabama in Birmingham, Birmingham, Alabama 35294*

Bruce W. Erickson (3), *The Rockefeller University, New York, New York 10021*

Charles T. Esmon (283), *Thrombosis/Hematology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104*

Paul F. Fitzpatrick (31), *Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802*

Shu Man Fu (213), *Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104*

Hao Fen-xi (135), *Institute of Microbiology, Academia Sinica, Beijing, China*

He Zhong-xiao (135), *Institute of Microbiology, Academia Sinica, Beijing, China*

Shabbir A. Khan (3), *The Rockefeller University, New York, New York 10021*

Vincent J. Kidd (235), *Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

Kung Tsu-hsun (223), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

Liang Chih-chuan (265), *Department of Molecular Biology and Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China*

Liang Dong-cai (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Lou Mei-zhen (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Meng Guang-zhen (135), *Institute of Microbiology, Academia Sinica, Beijing, China*

David S. Middlemas (199), *Braun Laboratories, California Institute of Technology, Pasadena, California 91125*

Nicholas Papadopoulos (31), *Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802*

Qi Zi-bei (265), *Department of Molecular Biology and Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China*

Qian Shi-jun (135), *Institute of Microbiology, Academia Sinica, Beijing, China*

Michael A. Raftery (199), *Braun Laboratories, California Institute of Technology, Pasadena, California 91125*

Betageri Rajashekhar (31), *Laboratory of Biochemistry, The Rockefeller University, New York, New York 10021*

Rao Zi-he (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Paul G. Schmidt (117), *Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104*

Jian-ping Shi (51), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

Ann M. Soderquist (175), *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

Robin Stackhouse (235), *Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

David Stuart (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

W. X. Tian (15), *Institute of Biophysics, Academia Sinica, Beijing, China*

Rosemary Todd (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Tsao Tien-chin (223), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

C. L. Tsou (15, 99), *Institute of Biophysics, Academia Sinica, Beijing, China*

Tu Guang-chou (297), *Kunming Institute of Zoology, Academia Sinica, Kunming, China*

Joseph J. Villafranca (31), *Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802*

Wan Zhu-li (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Wang Lin-fang (265), *Department of Molecular Biology and Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China*

Ying-lai Wang (51), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

Savio L. C. Woo (235), *Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030*

Xie Dian-lin (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Gen-jun Xu (51), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

Q. Z. Yao (99), *Institute of Biophysics, Academia Sinica, Beijing, China*

Ying Qilong (265), *Department of Molecular Biology and Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China*

You Jun-ming (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

James R. Zabrecky (199), *Braun Laboratories, California Institute of Technology, Pasadena, California 91125*

Alan Zell (65), *Departments of Biochemistry and Microbiology, Comprehensive Cancer Center and Institute of Dental Research, University of Alabama in Birmingham, Birmingham, Alabama 35294*

Joseph G. Zendegui (175), *Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232*

Zhang Ji-ping (157), *Institute of Biophysics, Academia Sinica, Beijing, China*

Zhang Mei-lin (265), *Department of Molecular Biology and Biochemistry, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China*

Fu-kun Zhao (51), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

K. Y. Zhao (15), *Institute of Biophysics, Academia Sinica, Beijing, China*

Zhu Shang-quan (185), *Shanghai Institute of Biochemistry, Academia Sinica, Shanghai, China*

Preface

This volume marks the second bilateral conference between the Peoples Republic of China and the United States dealing with *Proteins in Biology and Medicine.* The conference was held June 11–13, 1983 in Oklahoma City, Oklahoma, and dealt with several aspects of protein chemistry to which both Chinese and American scientists have made important contributions. Both the presentations and the resulting papers underscore the continued importance of research on proteins which has been clearly enhanced by the technologies of recombinant DNA analysis and monoclonal antibodies.

The meeting was held under the auspices of the Oklahoma Medical Research Foundation and was cosponsored by the Chinese Academy of Science and the Chinese Academy of Medical Science. The editors gratefully acknowledge the contribution of Dr. William G. Thurman, President of the Oklahoma Medical Research Foundation, and Professor Wang Ying-lai, past President of the Shanghai Institute of Biochemistry and the Shanghai Branch of the Chinese Academy of Sciences, who were responsible in large part for bringing this endeavor to fruition. The invaluable support of the staff of the Oklahoma Medical Research Foundation was also essential for the success of the conference as were the scientific contributions of the American and Chinese delegations.

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Structure and Function Relationships of Proteins

SYNTHETIC MODELS OF THE METASTABLE BINDING SITES OF ALPHA-2 MACROGLOBULIN AND COMPLEMENT COMPONENTS C3 AND C4

Bruce W. Erickson[1]
Shabbir A. Khan

The Rockefeller University
New York, New York

The complement system of serum proteins comprises a major element of the immune defense against infection by microorganisms. Complement components C3 and C4 facilitate the lysis and phagocytosis of immune complexes. Key steps in this process are the covalent attachment to immune complexes of C3 and C4 through their metastable binding sites (Tack *et al.*, 1980; Harrison *et al.*, 1981). Alpha-2 macroglobulin, a serum protein that inhibits a large variety of proteases, covalently attaches to a protease through a similar metastable binding site (Swenson & Howard, 1979, 1980; Sottrup-Jensen *et al.*, 1980a,b). These three serum proteins contain a common pentapeptide segment (Gly-Cyx-Gly-Glu-Glx) at their metastable binding sites. The gamma-carbonyl group of the Glx residue is chemically activated to nucleophilic attack and the beta-thiol of the Cyx residue is either sterically inaccessible or covalently masked.

STRUCTURES PROPOSED FOR THE METASTABLE BINDING SITE

Two chemical structures proposed for the metastable binding site are illustrated in Figure 1. Structure *A*,

[1]*This research was supported by U.S. Public Health Service grants AI 18362 awarded by the National Institute of Allergy and Infectious Diseases, DHHS, and CA 28571 and CA 33168 awarded by the National Cancer Institute, DHHS.*

which contains an internal residue of pyroglutamic acid (Glp, 5-oxoproline), was initially proposed to account for the spontaneous fragmentation of the metastable binding site (Howard *et al.*, 1980; Swenson & Howard, 1980; Howard, 1981). Under denaturing conditions, all three proteins undergo spontaneous fragmentation by cleavage of the Glu-Glx peptide bond. In the case of alpha-2 macroglobulin, one fragment bears an N-terminal Glp residue (Howard *et al.*, 1980). Structure *A* shows Cyx as Cys and Glx as Glp. For structure *A* to represent the metastable binding site, the Cys thiol group would have to be inaccessible to the thiol reagents. Alternatively, structure *B* shows Cyx and Glx as novel variants of Cys and Glu in which their side chains are covalently joined by a thioester bond (S-C=O). The gamma-carbonyl group of the Glx residue in structure *B* is activated because it is a thioester carbonyl group. In turn, the thiol group of the Cyx residue in structure *B* is covalently masked by being bound to the thioester carbonyl group.

We have synthesized and examined the chemistry of cyclic hexapeptide models of these metastable binding sites (Khan & Erickson, 1981, 1982; Erickson & Khan, 1983; Erickson *et al.*, 1983). Mercapto lactam *1* and thiolactone *2* of Figure 1 mimic the proposed structures *A* and *B*, respectively. In particular, the linear amino acid sequence present in these peptide models, Gly-Cyx-Glu-Glu-Glx-Asn, is also present at the metastable binding sites of both C3 and alpha-2 macroglobulin.

INTERCONVERSION OF THE SYNTHETIC PEPTIDE MODELS

Under physiologic conditions (phosphate-buffered saline, pH 7.3, 37°C), mercapto lactam *1* and thiolactone *2* exist in dynamic equilibrium (Figure 2). Since their ratio at equilibrium is *1/2* = 11, lactam *1* is about 1.5 kcal/mol more stable than thiolactone *2* (Khan & Erickson, 1982). Thus, the equilibrium mixture contains 92% of *1* and 8% of *2*. Part of the chemistry of the metastable binding sites can be explained by the interconversion of protein structures *A* and *B*. The lactam ring of *A* may be important in the biosynthesis of the thiolactone ring of *B*. Upon activation or denaturation of the protein, the thiolactone could either bind covalently to a receptive surface (Law *et al.*, 1979) or isomerize to the lactam, which could undergo spontaneous fragmentation.

On the basis of studies of space-filling molecular models, Davies and Sim (1981) have speculated that the side-

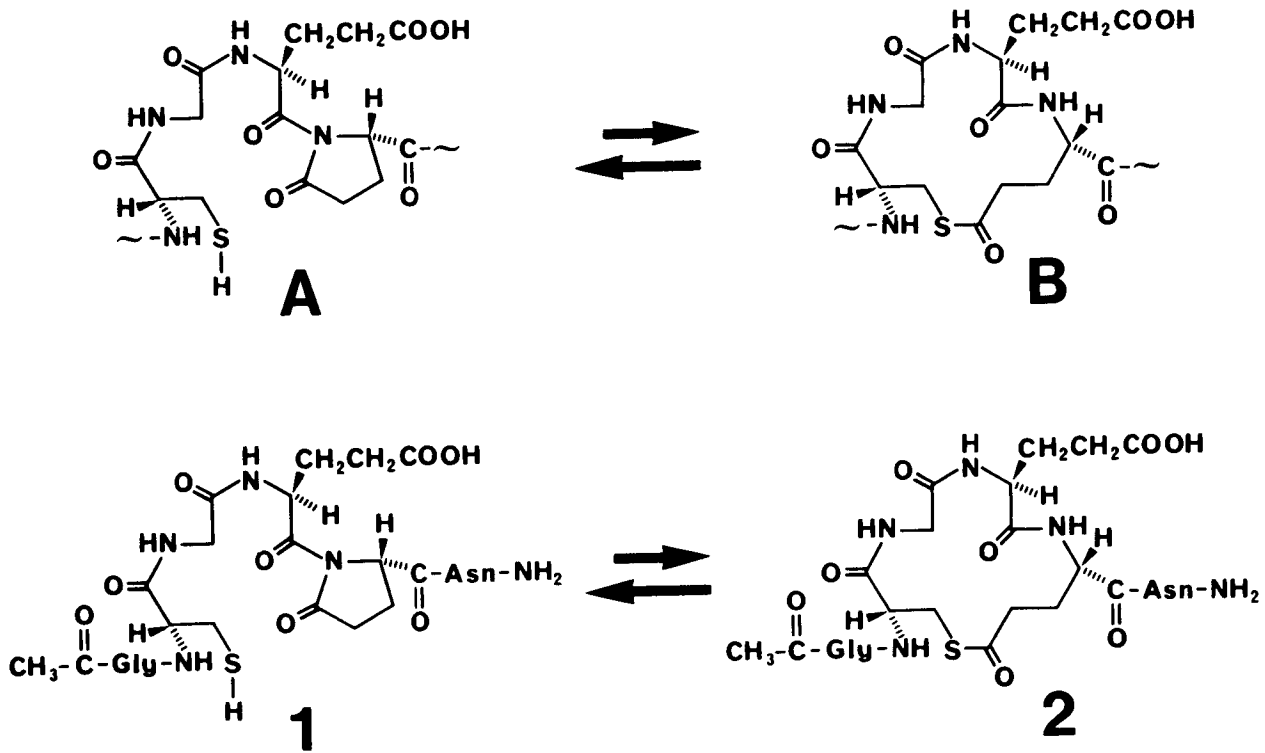

FIGURE 1. Structures proposed for metastable binding sites of alpha-2 macroglobulin and the complement components C3 and C4. Upper, *proposed chemistry of the protein sites which involves interconversion of 5-membered lactam* A *and 15-membered thiolactone* B. Lower, *synthetic hexapeptide models of these sites. The 5-membered lactam* 1 *mimics protein structure* A *and the 15-membered thiolactone* 2 *models protein structure* B.

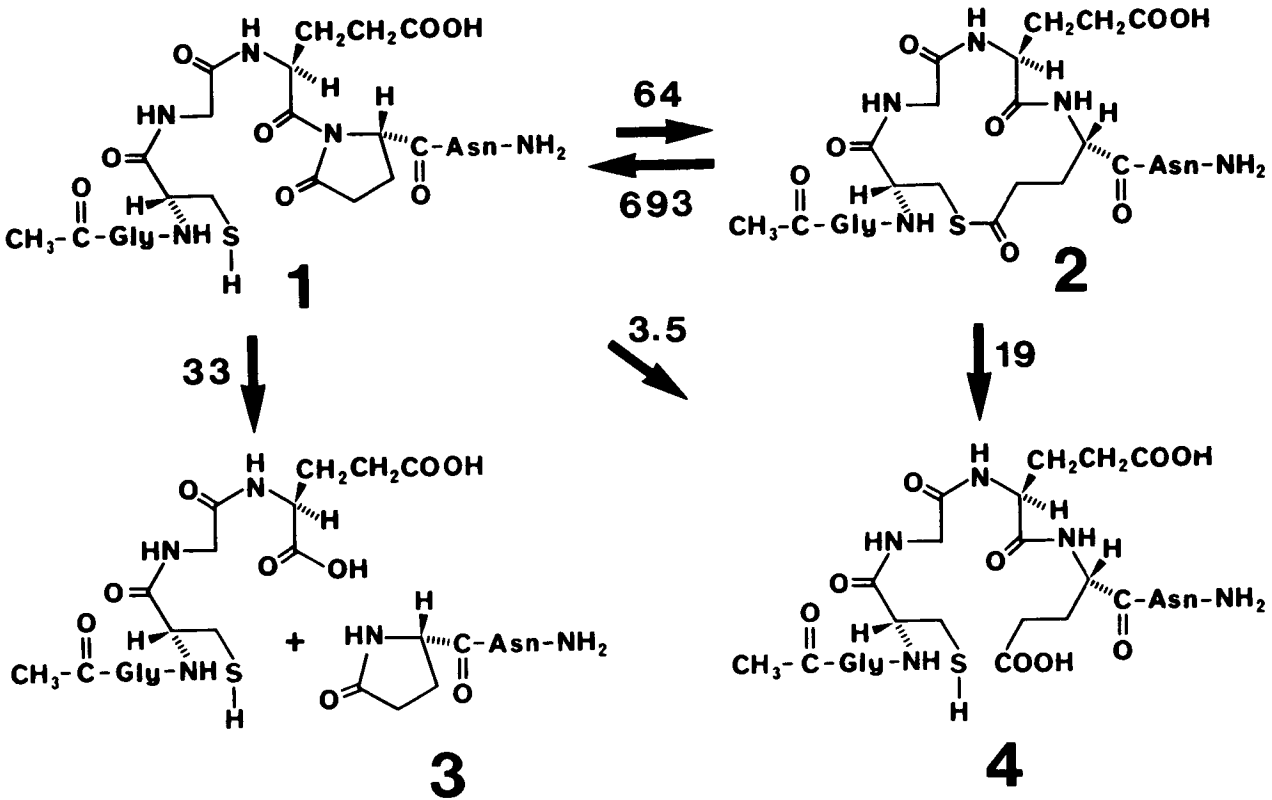

FIGURE 2. Chemistry of the Glu lactam 1 *and the Glu thiolactone* 2*. The apparent first-order rate constants (times* 10^{-6} s^{-1}*) for the competitive isomerization and hydrolysis reactions (phosphate-buffered saline, pH 7.3, 37°C) are shown beside the arrows.*