ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 303

# IMMUNOBIOLOGY OF PROTEINS AND PEPTIDES VI

## Human Immunodeficiency Virus, Antibody Immunoconjugates, Bacterial Vaccines, and Immunomodulators

Edited by M. Zouhair Atassi

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Edited by

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PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging-in-Publication Data

International Symposium on the Immunbiology of Proteins and Peptides (6th : 1990 : Scottsdale, Ariz.)
Immunobiology of proteins and peptides VI : human immunodeficiency virus, antibody immunoconjugates, bacterial vaccines, and immunomodulators / edited by M. Zouhair Atassi.
p. cm. -- (Advances in experimental medicine and biology ; v. 303)
"Proceedings of the Sixth International Symposium on the Immunobiology of Proteins and Peptides, held October 26-30, 1990, in Scottsdale, Arizona"--T.p. verso.
Includes bibliographical references and index.
ISBN 0-306-44038-5
1. AIDS (Disease)--Immunological aspects--Congresses. 2. HIV (Viruses)--Congresses. 3. HIV antibodies--Congresses. 4. Antibody-drug conjugates--Congresse. 5. Bacterial vaccines--Congresses. 6. Radioimmunotherapy--Congresses. I. Atassi, M. Z. II. Title. III. Series.
[DNLM: 1. Adjuvants, Immunologic--congresses. 2. Antibodies, Monoclonal--Immunology--congresses. 3. Bacterial Vaccines--immunology--congresses. 4. HIV--immunology--congresses. W1 AD559 v. 303 / QW 166 1613i 1990]
QR201.A37I57 1990
616.97'92079--dc20
DNLM
for Library of Congress 91-31837
CIP

Proceedings of the Sixth International Symposium on the
Immunobiology of Proteins and Peptides,
held October 26-30, 1990,
in Scottsdale, Arizona

ISBN 0-306-44038-5


A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013



Printed in the United States of America

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## PREFACE

The articles in this volume represent papers delivered by invited speakers at the 6th International Symposium on the Immunobiology of Proteins and Peptides. In addition, a few of the abstracts submitted by participants were scheduled for minisymposia and some of the authors, whose presentations were judged by the Scientific Council to be of high quality, were invited to submit papers for publication in this volume.

This symposium was established in 1976 for the purpose of bringing together, once every two or three years, active investigators in the forefront of contemporary immunology, to present their findings and discuss their significance in the light of current concepts and to identify important new directions of investigation. The founding of the symposium was stimulated by the achievement of major breakthroughs in the understanding of the immune recognition of proteins and peptides. We believed that these breakthroughs will lead to the creation of a new generation of peptide reagents which should have enormous potential in biological, therapeutic and basic applications. This anticipated explosion has in fact since occurred and many applications of these peptides are now being realized.

The sixth symposium was devoted to four major areas: **Human immunodeficiency virus, antibody immunoconjugates, bacterial vaccines and immunomodulators**. In this volume, many important papers will deal with various aspects of structure and biology of HIV and SIV, the expression and regulation of their genes and the immunology of their envelope proteins. Antibody immunoconjugates have become an important tool for specific targeting of drugs and radioisotopes in chemotherapy and radioimmunotherapy of certain malignancies. Papers by several leading investigators deal here with recent advances in this important field. Manipulation of the immune system by immunodulators, or by other strategies, is perhaps one of the most promising applications of the advances in immunology to disease therapy. Many important papers deal with designs and applications of vaccines against selected bacterial agents. To achieve an intelligent effective design of a vaccine, it is crucial to know details of the humoral and cellular immune responses against the infectious organism. How to maximize a required antibody or T-cell response or to reduce its magnitude is often desired in the design of an immunological defense strategy. What is the best means of delivery? How can tolerance be achieved in certain cases? These and other important questions that need to be appreciated in the design of vaccines are discussed in this volume.

Finally, I should like to express, on behalf of the organization, our gratitude to our sponsors whose generous support made this conference possible.

M. Zouhair Atassi

CONTENTS

# STRUCTURE AND FUNCTION IN RECOMBINANT HIV-1 gp120 AND SPECULATION ABOUT THE DISULFIDE BONDING IN THE gp120 HOMOLOGS OF HIV-2 AND SIV

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## INTRODUCTION

The envelope glyco-proteins of the primate immunodeficiency viruses (HIV-1, HIV-2 and SIV) have been the objects of intense study since their discovery. The major envelope glycoprotein (gp120 in HIV-1) is of particular interest because it mediates the attachment of the virus to susceptible cells via the CD4 molecule[1,2], it contains most of the important epitopes for neutralization of the virus by antibodies[3,4,5], it plays an important role in the process by which the viral and host cell membranes fuse and the viral capsid gains access to the cytoplasm[6,7], and its sequence variability is central to the ability of the virus to adapt to and escape the protective immune response of the host organism[8]. Complete understanding of these processes requires an understanding of the molecular structure of gp120 in detail. Such structural information has proven to be difficult to obtain because of the large size of gp120 (approximately 480 amino acids), its high degree of glycosylation (approximately 50% by weight), the high degree of heterogeneity of the oligosaccharides on the molecule, and the scarcity of material available for analysis.

The scarcity of gp120 protein for structural analysis has largely been overcome by its production in recombinant mammalian cells[9]. Because recombinant gp120 (rgp120) is secreted from these cells with functional properties identical to those of gp120 produced by virally infected cells[10], it presumably has structural properties very similar to those of the viral protein. Mammalian cells, such as the CHO cells used for the production of rgp120, also glycosylate proteins in a manner similar to that expected for gp120 produced by virally infected cells and offer a degree of

*Immunobiology of Proteins and Peptides VI*
Edited by M.Z. Atassi, Plenum Press, New York, 1991

confidence that the structural attributes of rgp120 produced in them are also representative of the viral protein.

The ultimate goal of x-ray chrystalographic analysis of rgp120 has proven to be very dificult to achieve at least in part because of the extreme heterogeneity of the oligosaccharide moieties on the protein. A determination of the disulfide bonding pattern and the type of oligosaccharide at each of the potential N-linked glycosylation sites in rgp120 fron HIV-1 IIIB has however recently been reported[11]. In this paper we expand on the discussion of that analysis and extend its interpretations to some of the structural variants among the primate immunodefficiency virus gp120 homologs. We also use the HIV-1 gp120 data to predict the disulfide bonding pattern for the gp120 homolog of the HIV-2 isolate, ROD, and the SIV isolate, SIV-MM142.

## THE PRIMARY STRUCTURE OF rgp120-IIIB

The structural analyses were performed on two forms of rgp120 from the IIIB isolate of HIV-1 produced in recombinant CHO cells[11]. For ease of expression and purification both of these proteins were constructed as fusion proteins consisting of a portion of the Herpes Simplex Virus type 1 glycoprotein D (gD) fused to the truncated N-terminus of gp120. One of the forms of rgp120 (CL44) was composed of the N-terminal 27 amino acids of mature gD fused to amino acid 31 of mature gp120 and the other form (9AA) was composed of the N-terminal 9 amino acids of gD fused to amino acid 4 of gp120. As a consequence of the construction the rgp120 CL44 protein is missing the first cysteine residue (C24) encoded by the mature gp120 sequence, and therefore has one unpaired cysteine. Because of this the determination of the disulfide bonding pattern was done first with the 9AA protein and then partially confirmed with the CL44 protein. Characterization of the 24 potential N-linked glycosylation sites was performed with the CL44 protein. The purified proteins (non-reduced for the disulfide determinations or reduced and carboxymethylated for the oligosaccharide analyses) were analyzed by tryptic digestion, rpHPLC purification of the resulting peptide fragments and identification of the peptides by quantitative amino acid analysis and N-terminal sequencing. Further proteolytic and/or glycosidic digestion of specific peptides and re-purification by rpHPLC were required to assign all of the disulfide bonds in the peptide fragments. The type of oligosaccharide present (i.e., complex or high mannose) at each potential N-linked site was determined on the basis of susceptability to endo glycosidase H.

The results of the analyses, summarized in Figure 1, indicate that the 18 cysteines of the rgp120-IIIB protein are all disulfide bonded to form a series of five domains: two domains, each with one disulfide bond, and three domains each containing a more complex pattern of two or more disulfide bonds. No heterogeneity of the disulfide bonding pattern was detected other than the unpaired cysteine (C44) in the CL44 form of rgp120-IIIB. All of the 24 potential N-linked glycosylation sites were utilized, with mostly complex type

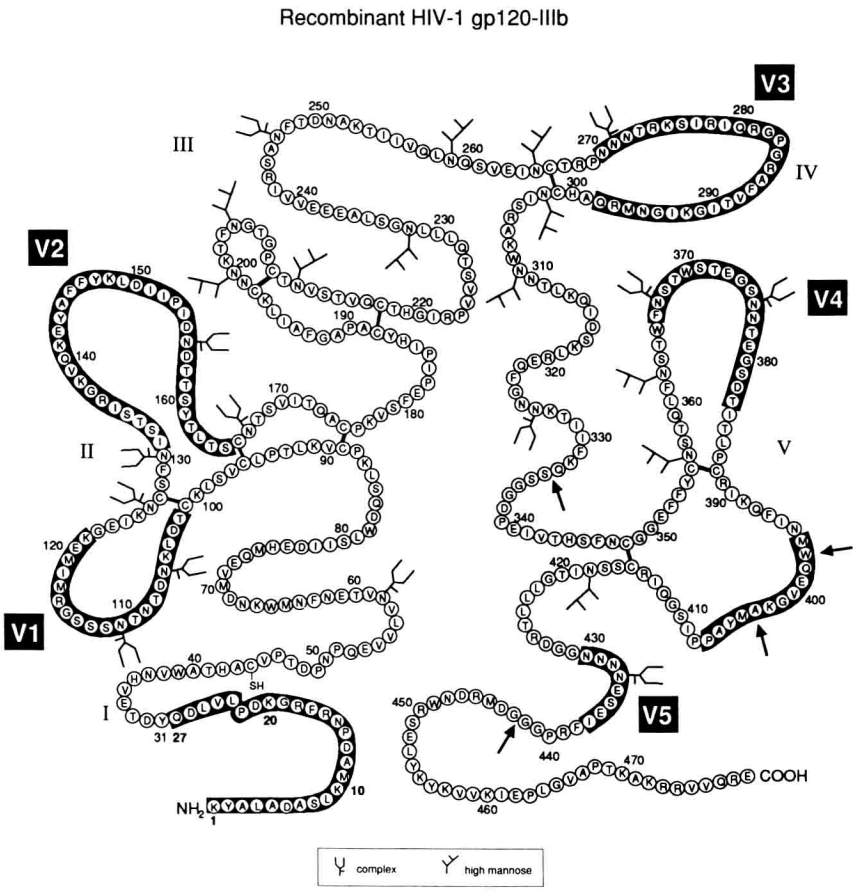


Figure 1. Model Of The Primary Structure Of Recombinant gp120 Of HIV-1 IIIB. This is a summary of the experimental work of Leonard, et al.[11] on the recombinant fusion protein CL44 expressed in CHO cells. The protein is composed of the N-terminal 27 amino acids of herpes symplex virus glycoprotein D (shaded) fused to amino acid 31 of gp120. The type of oligosaccharide structure present at each N-linked glycosylation site is indicated. The Roman Numerals refer to the five disulfide bonded domains and the boxed sequences accompanied by a boxed number refer to the hypervariable regions described by Modrow, et al.[8]. The arrows and the box around residues 396 to 407 designate the sites implicated in CD4 binding by mutagenesis studies[10, 13].

oligosaccharide structures at 13 of them and mostly high mannose or hybrid type structures at the remaining 11 sites. In general, the oligosaccharides at individual glycosylation sites were found, within the limits of detection, to be either completely resistant or completely susceptible to endo H. The only heterogeneity of this type detected at any of the N-linked sites was the presence of a trace amount of high mannose or hybrid type oligosaccharide at N246 instead of the predominant complex type structure. No O-linked oligosaccharides were detected.

## REGIONS INVOLVED WITH CD4 BINDING

One of the most studied functional attributes of gp120 is its binding to the HIV cellular receptor CD4. This interaction has been well characterized using rgp120 and a soluble recombinant form of CD4[12]. The majority of information about the portions of gp120 that interact with CD4 has come from mutagenesis studies and the mapping of the epitope of an anti-rgp120 monoclonal antibody that blocks CD4 binding[10,13]. This information is summarized in Figure 1. Deletion of residues 396 to 407 was reported by Lasky et al[10] to abolish CD4 binding by the resulting recombinant mutant gp120 protein. More specific mutagenesis within this region of A403 to D[10] and W397 to S, G, V or R[14] abolished CD4 binding by the mutant proteins and implied that these residues were critically important for the interaction. Short insertions between residues 333-334, 388-390 and 442-443 by Kowalski et al[13] similarly abolished CD4 binding. The epitope of the murine monoclonal antibody 5C2-E5, which blocks the binding of rgp120 to CD4, was mapped to residues 392-402[10]. Further evidence comes from a report that a proteolytic fragment of gp120 from residue 322 to near the C-terminus retains the ability to bind to CD4[15]. In summation, the currently available data suggest that the portions of gp120 that are involved with its interaction with the CD4 molecule are found at various sites between residues 320 and 450.

On a linear map of the gp120 sequence the regions implicated in CD4 binding show little relation to each other. However, the disulfide bonding pattern shows that all of these sites are associated with a discrete disulfide bonded domain, domain V in Figure 1. The five sites identified above are located in conserved sequences[16] either between C388 and C415 or along the "neck" of domain V upstream from C348 and downstream from C415.

## THE OLIGOSACCHARIDES OF rgp120

The structures of the carbohydrate moieties of gp120 have been determined for glyco-protein produced by virally infected H9 cells[17,18] and CL44 rgp120 produced in CHO cells[19]. A summary of the results of these analyses is presented in Table 1. A large proportion of the structures are of the high-mannose type which is sometimes associated with premature release of protein (e.g., by cell lysis) that has not completed the final stages of oligosaccharide processing. For CL44 rgp120 this is probably not the case as

the high mannose (i.e., endo H susceptible) structures were found to be localized preferentially at 11 of the 24 sites. In the event of significant cell lysis, a mixture of completely processed (complex type) structure and incompletely processed (high-mannose type) structure would be expected at all glycosylation sites. In the analyses of the oligosaccharides on rgp120 and secreted viral gp120 summarized in Table 1[17,19], 39% and 54% of the total released structures were of the high-mannose or hybrid type, respectively. In the study in which the type of structure at each individual site was determined[11] 11 of the 24 total sites, 46%, had high-mannose or hybrid type structures. In contrast, and as expected, the gp120 isolated from virally infected cells had a higher proportion, 62% and 83%, of these structures, probably due to release of incompletely processed protein. The sum of this data suggests that high-mannose and/or hybrid type oligosaccharide moieties are normal components of mature gp120, produced either by recombinant CHO cells or virally infected cells, and that they are not an artefact of cell lysis.

In CL44 rgp120 7% of the oligosaccharides have a hybrid type structure[19]. The 7% hybrid structures in CL44 could represent the summation of trace amounts of such structures at some or all of the glycosylation sites in the final product. Also, it is known that glyco-proteins expressed in CHO cells can be secreted with a mixture of high mannose and complex type oligosacharides at a particular glycosylation site[20]. However 4% of the total oligosaccharides present on rgp120 corresponds to 1 of the 24 total N-linked sites and could suggest that at one particular site on gp120 the final processed oligosaccharide is of the hybrid type.

Table 1. Types of Oligosaccharides On gp120

| | High Mannose | Hybrid | Complex |
|---|---|---|---|
| CHO rgp120 [19a] | 32% | 7% | 61% |
| H9 sgp120 [17] | 54%[b] | - | 46% |
| H9 cgp120 [17] | 83%[b] | - | 17% |
| H9 cgp120 [18] | 60% | 2% | 37% |

[a)]Mole % data summarized from the references in parentheses; CHO rgp120 is soluble recombinant gp120 expressed in CHO cells; H9 sgp120 and H9 cgp120 are soluble or cell-associated gp120, respectively, produced by HIV-1 IIIB infected H9 cells. [b)]Numbers represent total Endo H released oligosaccharide and, as such, are the sum of the high-mannose and hybrid mole percentages.

DISULFIDE BOND VARIANTS

The pattern of cysteines in gp120 IIIB is highly conserved among the HIV-1, HIV-2 and SIV isolate sequences compiled in the Los Alamos data base[16]. Of the published