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I. The Nature of Antigenicity

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INTRODUCTION

B. Cinader

We shall define antigenicity as the triggering of a lymphoid cell by a molecule and the subsequent capacity of the same type of molecule to combine with an elicited product of cellular differentiation. The term antigenicity thus includes two functions: immunogenicity i.e. the capacity to induce a specific immune response and antigenic specificity i.e. the capacity of a molecule (irrespective of its immunogenicity) to react specifically with a product of induced lymphoid cell differentiation.

The final manifestation of antigenicity is in the reactivity and specificity of antibody and of activated T cells i.e. in the molecular and cell effectors which are the end results of the triggering interaction. The antibody elicited by macromolecules serves to identify, the chemical composition and conformation of antigenic determinants (Arnon[1]). Furthermore, it may identify regions of the antigen which control biological activity, since antibody can block access to active sites by steric hindrance and can modify activity by distorting the conformation of sites[2]. The genetic markers of the antibody, particularly idiotypes, provide clues to the genetic controls which determine affinity, or which modify antigen-mediated affinity selection of B cell receptors (Nisonoff et al[1]).

Antigenicity does not depend, exclusively, on determinants which select appropriate Ig receptors of B cells and with which the antibody can combine. In fact, the antigen carries a set of signals for a series of additional events which regulate the immune response. These include differentiation, proliferation and interaction of different cell types. The signals reside primarily in the three-dimensional structure of the antigen and secondarily in its concentration and state of aggregation.

The structural requirements for cell triggering are distinct from those for cell cooperation. Rigidly spaced bifunctional antigens promote T-B cell cooperation, but not proliferation. Monofunctional antigens induce, in T cells, DNA synthesis and in B cell proliferation but not maturation to antibody forming cells (Goodman et al[1]). Separate structures on the antigen may invoke cooperative participation of macrophages[3]. Distinctive configurations of the antigen interact with B cell receptors for mitogens, and the response then appears to bypass the requirement for determinants which invoke T cell help[4]. Aggregation directs the antigen to a pathway which, via a relatively radiation resistant accessory cell, induces antibody-formations. The same concentration of the same but aggregate-freed antigen may induce unresponsiveness in T and B cells[5 & 6]. T cells seem to have a lower threshold for tolerance induction than do B cells. Absence of activated T cells and failure to generate "on signals" in B cells appear to result in "off signals" if saturating quantities of antigen are present (G.F. Mitchell[7]). Macromolecules which cannot be metabolized, induce B cell unresponsiveness by

persistance on the membrane, presumably, because combination of the receptor with undigestable antigens prevents the normal cycle of membrane clearance (E.R. Unanue and K.A. Ault[7]). There are separate signals for delayed hypersensitivity, antibody formation and tolerance . Parish[1] has shown that polymerized flagellin does not induce delayed hypersensitivity,whereas monomeric flagellin is very potent in this respect - as is heavily acetoacetylated flagellin. Acetoacetylated flagellin is unable to induce antibody formation, but induces tolerance. The ability to provoke increased delayed hypersensitivity and tolerance are not necessarily coupled. For instance, succinylated flagellin provokes delayed hypersensitivity, but not tolerance.

Whether a signal can be "heard" by lymphoid cells of a given individual, i.e. whether it can be received at the membrane and/or transmitted to the synthetic apparatus, depends on various components of each individual's genotype. The most widely studied of the multiple controls of dominant inheritance resides in the major histocompatability autosomal region (Ir, MLR loci, effector cell stimulator locus). Recessive inheritance of immunological responsiveness can be controlled by tolerance to autologous molecules[8,9,10]. This "steering mechanism" might also control dominant inheritance of the Ir type through a regulatory gene for autologous antigens or for suppressor cells.

Antigenicity thus results from the interplay between a set of regions on the surface of a foreign macromolecule and a set of genes, controlling (i) the presence and identity of lymphoid membrane receptors, and (ii) the signals which are transmitted when the receptor is activated. Considerable advances have been made in identifying some of the components of this interaction. When the problem is finally resolved, Immunology may cease to be one of the most intriguing and challenging areas of modern biology - but that day is in the distant future.

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CONFORMATION AND PHYSICO-CHEMICAL FACTORS INFLUENCING ANTIGENICITY

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INTRODUCTION

The understanding and elucidation of the role of various physico-chemical parameters which affect antigenicity has been the paramount goal of the studies on the chemical basis of antigenicity. In this context one should realize that the term antigenicity comprises two independent capacities, which should be clearly distinguished from each other. These are immunogenicity, namely, the ability to provoke an immune response regardless of its specificity, and antigenic specificity, which defines the competence of a molecule to react specifically with an antibody, without any presumption that it would be able by itself to elicit formation of such antibody.

In studies of this kind, two different approaches have been employed. In the first, the analytical approach, the starting point is an immunogenic molecule, fragments and chemical derivatives of which are screened for either immunogenicity, or specificity, i.e., the capacity to react with antibodies to the intact immunogen. There are many examples of studies of this type, which have perhaps found their culmination in the essentially complete antigenic mapping of a globular protein, myoglobin[1]. The alternative approach has been the synthetic route, namely, use of non-antigenic starting units for building synthetic antigens. The advantage of this approach is that, once the immunogenicity of one synthetic material has been unequivocally demonstrated, many analogs may be prepared and tested. Since the chemistry of these analogs is known and controlled, they can lead to an understanding of the role played by various physicochemical parameters in conferring antigenic activity. Indeed, studies of synthetic antigens over the last two decades have provided a large body of information regarding the role of shape, size, composition and electrical charge of the macromolecule, as well as of the locus of the antigenic determinant in the molecule, and of the optical configuration and steric conformation[2].

A combination of these two approaches, namely, the chemical synthesis of an immunologically active protein fragment and analogs of it, should lead to an elucidation of the physicochemical parameters involved in antigenicity of native proteins.

The main topic of this presentation is the decisive role played by spatial conformation in determining antigenicity and particularly antigenic specificity of proteins and protein-like molecules. In this respect, the influence of conformation on both the humoral immune

response and the cellular recognition of antigens will be considered.

ROLE OF CONFORMATION IN ANTIGENICITY OF PROTEINS

The steric conformation of antigenic determinants seems of special importance in view of our knowledge that denaturation of native proteins, or unfolding of their polypeptide chain, results in a drastic change in their antigenic properties. The denatured or unfolded proteins are still immunogenic, but their antigenic specificity is totally different from that of the corresponding native proteins[3]. One can distinguish, therefore, in protein and polypeptide antigens, between "sequential" determinants and "conformational" determinants[4]. Sequential determinants are those due to a particular amino acid sequence in a random coil form, and antibodies specific to them will react also with a peptide of identical or similar sequence. Conformation-dependent determinants result from the steric conformation of the molecule, due to its secondary, tertiary and even quaternary structural features. They would include those determinants composed of amino acid residues which even if remote in the unfolded polypeptide chain, occupy juxtapositions in the native structure.

Examination of the three-dimensional structure of a number of globular proteins reveals that they contain short sequences of adjacent amino acids whose side chains are partially or fully exposed on the surface of the protein. Consequently, these could exist as sequential determinants. However, in practice it appears that when such short peptide fragments of a protein were shown to interfere with the interaction of the native protein with its antibodies, this capacity is often due to the fact that the peptides are induced by the antibodies to refold into the structure that they hold in the native protein. There are only a few clear-cut cases where sequential determinants were demonstrated, such as the terminal segments of collagen, or silk fibroin. Otherwise, it seems that antibodies to native globular proteins are directed mostly, and in several cases exclusively, against conformation-dependent determinants. This can be best exemplified by several well-documented systems in which conformational determinants were allocated and identified:

a. Myoglobin. This protein, of molecular weight 18,400, has no disulfide bridges, and its structural integrity is due mainly to the high contents of its tertiary structure. Immunologically active fragments of the molecule, obtained by various methods of cleavage, all occupied regions of "corners" in the three dimensional configuration of the molecule[1]. Examination of a space filling molecular model reveals that these corners of the polypeptide chain coincide with the more exposed areas, which, due to the folding of the molecule are held in a fixed conformation. Most of the antigenic activity of myoglobin may, therefore, be attributed to its primary and secondary structures, but, the tertiary structure, brought about by the ferrihaem group, has also been implicated in the antigenicity. This was indicated by

the capacity of antibodies to the haem-containing protein to distinguish between the apoprotein and metmyoglobin, and by the finding that antibodies to the apoprotein are able to release the haem group from the metmyoglobin[5].

b. Staphylococcal Nuclease. This is another example of a globular protein devoid of disulfide bridges, in which the folded structure is held together mainly by hydrophobic interactions, and yet possess conformational specificity. Fragmentation of the protein by cyanogen bromide or trypsin allowed the antigenicity to be correlated with three regions of the molecule, namely, residues 18-47, 99-126 and 127-149. However, when antibodies formed by administration of the immunogenic fragment 99-149 were compared with antibodies isolated from antisera to the intact protein, by using the fragment attached to Sepharose as an immunoadsorbent, it was shown that the two types of antibodies, both directed apparently to the same 50-amino acid C-terminal region of the molecule, revealed marked differences. Whereas the anti-whole protein recognized only the native form of the fragment, the antibodies elicited by the fragment recognized only the random unfolded protein[6]. It was thus concluded that although nuclease has a low helix content, and lacks any disulfide bonds, its structural conformation influences its antigenic determinants.

c. Collagen and Synthetic Collagen-like Copolymer (Pro-Gly-Pro)$_n$. A better understanding of the role of conformation in the antigenicity was achieved in recent years by building appropriate synthetic models and analyzing their immunochemical properties. One example for this approach is a collagen-like synthetic copolymer. Collagen is a protein which, except for the 10-15 N-terminal amino acid residues, comprises highly repetitive amino acid sequence, with the triplet Gly-Pro (or Hyp)-X being the repeating unit, arranged in a unique triple helical structure[7]. Immunization with native collagen leads mainly to antibodies directed to the N- and C-terminal non-helical regions which show interspecies differences. The presence of conformational determinants in collagen was indirectly proven by the use of a synthetic approach: A synthetic periodic polypeptide (L-Pro-Gly-L-Pro)$_n$, which was shown to have a collagen-like triple-helical structure, was found to be immunogenic in guinea pigs and rabbits. Immunization with this copolymer elicited antibodies that cross-reacted with collagens of several species[8]. On the other hand, a random copolymer of a similar composition, (poly-L-Pro^{66}Gly^{34}), did not cross-react with collagens. Hence, the polymer of ordered sequence reacted immunologically with the various collagens by virtue of the triple-helix conformation which is common to both substances, and serves as the major antigenic feature.

A UNIQUE CONFORMATIONAL DETERMINANT OF LYSOZYME AND ITS SYNTHETIC ANALOGS

The immunological specificity of lysozyme is almost entirely dependent on its three dimensional native conformation, as has been implied, on the one hand, by its total lack of cross-reactivity with its unfolded, reduced and carboxymethylated derivative, and on the other hand, by the isolation of several fragments of the native molecule which retain immunological activity and represent independent antigenic determinants. One such isolated fragment, consisting of the amino acid sequence 60-83, and containing an intrachain disulfide bond, was denoted "loop"[9]. The location of this peptide in the three dimensional structure of lysozyme is shown in fig. 1. Antibodies specific exclusively to this region were prepared either by selective isolation from anti-lysozyme serum on a "loop" immuno-

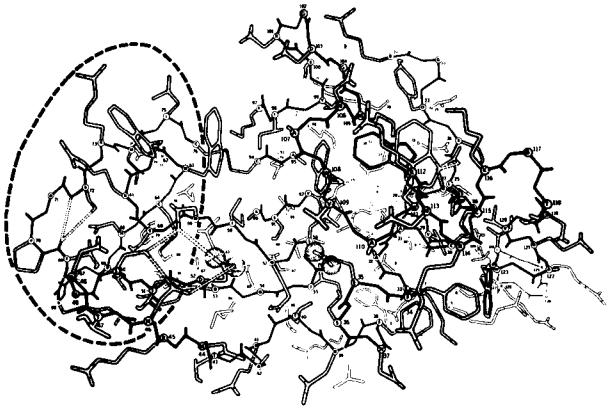

Fig. 1. Backbone and sidechains of lysozyme. (From the Atlas of Protein Sequence and Structure, M.O. Dayhoff, Ed). The loop region is encircled.

adsorbent, or by immunization with a conjugate containing the loop attached to a synthetic carrier. These anti-loop antibodies were reactive efficiently with native lysozyme, as well as with the isolated loop peptide derived from it, but not at all with the open-chain loop peptide in which the disulfide bond was disrupted by reduction and alkylation. The specificity of these antibodies was investigated by several sensitive techniques, including a fluorometric method, using a loop derivative in which a fluorescent chromophore (dansyl group) was attached. These data, shown in fig. 2, indicate that the loop region is a conformation-dependent antigenic determinant, and that its specificity is dictated by a spatial structure similar to that which it assumes in the native lysozyme molecule.