Structural Aspects of Recognition
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THE CONFORMATION OF ANTIGENIC DETERMINANTS OF INSULIN AND H-2 GENE CONTROL OF THE IMMUNE RESPONSE OF T LYMPHOCYTES

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ABSTRACT

The magnitude of the immune response of T lymphocytes to antigenic determinants of ungulate insulins is controlled by the products of H-2 genes. H-2 genes do not appear to code for the lymphocyte receptors that directly recognize antigenic determinants, but by a yet unknown mechanism select the antigenic determinants which are forbidden or permitted to the immune system.

We carried out experiments designed (1) to characterize the behavior of H-2 gene products in selecting antigenic determinants, and (2) to define the conformation of the determinants. The aim was to learn the structural correlates of H-2 gene control of recognition by T lymphocytes.

Based on the crystallographically solved structure of pork insulin, the close homology of all the ungulate insulins and the amino acid substitutions, we built models of the different insulins. We analyzed the likely conformations of the antigenic determinants, and proposed structural specificities accounting for the immunologic phenomena observed.

1. INTRODUCTION

The immune response towards a foreign substance that penetrates into the organism is usually directed against certain regions on the substance. These regions are known as 'antigenic determinants.' The three-dimensional structure of an antigenic determinant is recognized by complementary receptors found on clones of T and B lymphocytes.

Even though the precise molecular events are not defined, there is reason to believe that recognition of soluble protein antigens by the immune system requires an initial uptake of the antigen by

Table 1. Amino acid substitutions of insulins

Insulin	Substituted residues					
	A chain loop			Non-loop		
	8	9	10	A4	B3	B30
Mouse	Thr	Ser	Ile	Asp	Lys	Ser
Pork	Thr	Ser	Ile	Glu	Asn	Ala
Beef	Ala	Ser	Val	Glu	Asn	Ala
Sheep	Ala	Gly	Val	Glu	Asn	Ala

(Taken from Atlas of Protein Sequence and Structure; Dayhoff, 1972)

macrophages. The macrophages process the antigen and 'present' antigenic determinants to thymus-dependent T lymphocytes. T lymphocytes which are known as helper T lymphocytes can signal another class of cells, the B lymphocytes, and aid them in the induction of antibody production to the antigen.

For successful transmission of an antigenic signal between macrophages and T lymphocytes, the cells must share genetic identity at some portion of the major histocompatibility complex (MHC). The MHC of the mouse, termed the H-2 complex, is a cluster of genes positioned in the middle of chromosome 17, whose products are defined by different alleles (Klein et al., 1978; Klein, 1979).

It was found in many cases that the immune response to various antigenic determinants is controlled by specific genes in the MHC. Such genes are defined as immune response (Ir) genes. One of the main roles of Ir gene products is to direct the attention of the immune response to particular antigenic determinants from among all the conformational entities present on certain antigens. Ir genes do not code for the receptors of lymphocytes that recognize antigenic determinants, but influence which receptors will be activated to selected antigenic determinants. Thus, the immune response to a particular antigenic determinant is mediated by macrophages, T and B lymphocytes, under the direction of Ir gene products.

Table 2. Immune reactivities and cross-reactivities define antigenic determinants on insulins

Determinant	Cross-reactivities of response to insulins		
	Pork	Beef	Sheep
A chain loop (A8, A9, A10)			
Variants: A9 Ser=Gly	-*	+†	+
A9 Ser	-	+	-
A9 Gly	-	-	+
Non-loop (A4, B3, B30)	+	+	+

*Relatively low or no response.
†Relatively high response.

An unanswered question is how Ir genes work to select particular antigenic determinants. One way to approach this question is studying the immune response to small protein antigens whose amino acid sequences and 3-dimensional structures are known. Insulin is a useful antigen for this purpose. Nature provides us with a large number of evolutionary variants of insulin that differ from one another by substitution of a small number of amino acids.

Table 1 shows the amino acid substitutions of pork, beef and sheep insulins, as compared to mouse insulins I and II. The two positions that distinguish between mouse insulins I and II are B9 (Pro in I and Ser in II) and B29 (Lys in I and Met in II). Since at least one of the mouse insulins is identical with the three ungulate insulins at these two positions, the substitutions probably do not contribute to foreign determinants. The three ungulate insulins all differ from the mouse insulin by common substitutions at A4, B3 and B30, which are outside the A chain loop. In addition, beef and sheep insulins differ from mouse insulin in part of a loop present in the A chain (A8-A10) (Markussen, 1971; Dayhoff, 1972). Except for those substitutions

the other parts of ungulate insulins are identical to mouse insulin and may be considered to represent mouse self-determinants.

In general, animals are tolerant to their own proteins, otherwise they would suffer from autoimmune diseases. Therefore, we assume that antigenic determinants recognized by T lymphocytes on ungulate insulins are those that differ from mouse insulins, since only such determinants can be recognized as non-self.

2. IMMUNOLOGICAL ANALYSIS OF ANTIGENIC DETERMINANTS OF INSULIN

What are the structural differences between the different foreign antigenic determinants found on various insulins, and how are these structural differences perceived by Ir gene products?

In order to approach these questions, we studied the response of mouse T lymphocytes to various ungulate insulins (Cohen and Talmon, 1980). Strains of mice differing only at their H-2 genes were immunized with insulins emulsified in Freund's complete adjuvant. After 14 days, the draining lymph nodes were removed and suspensions of lymphocytes were challenged in an in vitro secondary response against various ungulate insulins. The response of T lymphocytes was measured by ^{3}H-thymidine incorporation into the DNA of stimulated cells (Cohen et al., 1979).

Table 3. Response of H-2^{d} mice to immunization with pork, beef and sheep insulins

Immunization of H-2^{d} mice (Balb/c) with insulin	In vitro proliferative response		
	Background cpm	Δ cpm in the presence of insulins	
Pork	3,500	Pork	22,507
		Beef	21,091
		Sheep	19,736
Beef	3,752	Beef	68,682
		Sheep	56,813
		Pork	532
Sheep	19,541	Sheep	143,744
		Beef	111,112
		Pork	0

Δ cpm = cpm of tested group minus spm of background (w/o insulin).

Table 2 shows that measuring the cross reactivities of the immune responses to pork, beef and sheep insulins, we are able to define immunologically different antigenic determinants on the insulin molecule. For example, an immune response that failed to distinguish between the three ungulate insulins would localize an antigenic determinant(s) to the non-loop region in which all three insulins have identical substitutions. In contrast, a response to beef and/or sheep but not to pork insulin would localize an antigenic determinant to the A chain loop, since sheep and beef differ from pork and mouse at the loop. We can conceive of three variations of an immune response to the A chain loop: (a) No distinction between sheep and beef insulins indicating immunologic identity between A9 Ser and A9 Gly; (2) a higher response to beef insulin (A9 Ser), and (3) a higher response to sheep insulin (A9 Gly).

$H\text{-}2^d$ mice were immunized with beef, sheep and pork insulins and their response to the three ungulate insulins was measured (Table 3). Cross reaction between pork, beef and sheep insulins was found when $H\text{-}2^d$ mice were immunized with pork insulin. Since these three ungulate insulins share common amino acid substitutions outside of the A chain loop, we can conclude that immunization with pork insulin led to a response to non-loop antigenic determinants. In contrast, there was no response to pork insulin when these $H\text{-}2^d$ mice were immunized either with beef or with sheep insulin. This indicates that the response under those circumstances was directed towards the A chain loop determinant. The presence of a foreign A chain loop determinant prevented the immune response against non-loop determinants. Can this dominance of one determinant over another be explained by a functional interaction between the A chain loop and a non-loop structure? Or perhaps the loop and non-loop substitutions together form a single antigenic determinant?

Another kind of relationship between antigenic determinants was found in $H\text{-}2^b$ and $H\text{-}2^k$ mice. Such mice could respond to immunization with beef ($H\text{-}2^b$) or sheep ($H\text{-}2^k$), but not to pork insulin (Table 4). Therefore, $H\text{-}2^b$ or $H\text{-}2^k$ mice could only respond to an A chain loop determinant. However, unlike $H\text{-}2^d$ mice which responded equally well to either beef or sheep insulins, $H\text{-}2^b$ and $H\text{-}2^k$ mice distinguished between these two insulins that differed only at A9. $H\text{-}2^b$ responded

Table 4. Response of H-2^k and H-2^b mice to immunization with beef and shee insulin

Strain	H-2 genotype	Immunization in vivo	Background cpm	In vitro proliferative response (insulins Δ cpm: test minus background	
C3H.DiSn	k	Sheep	9,403	Sheep	18,220
				Beef	3,053
				Pork	0
		Beef	20,340	Beef	0
				Sheep	20,066
				Pork	0
C3H.Sw	b	Beef	16,964	Beef	227,146
				Sheep	63,174
				Pork	3,284
		Sheep	9,785	Sheep	0
				Beef	16,035
				Pork	0

Mice were immunized with either beef or sheep insulin, as described in Table 2 and the proliferative response of the lymphocytes was measured against beef, sheep and pork insulins.

only when immunized with beef (A9 Ser) while H-2^k responded only when immunized with sheep (A9 Gly) insulin.

However, we found that the "forbidden" A chain loop determinant that was not immunogenic for a response against itself could still cross immunize mice for a response against the "permitted" variant of the A chain loop (Table 4). For example, when H-2^b mice were immunized with sheep insulin they did not respond in vitro to sheep insulin but did respond to beef insulin that had not been injected. Likewise, H-2^k mice when immunized with beef insulin did not respond to beef insulin but did respond in vitro to sheep insulin. Therefore, we observed a kind of "mimicry" between A9 Ser-Gly variants of the A chain loop. H-2^b mice responded to A9 Gly as if it were A9 Ser, while H-2^k mice responded to A9 Ser as if it were A9 Gly. Since this mimicry is related to the H-2 genotype of the mice, it is likely to reflect the function of H-2 gene products in selecting antigenic determinants. What is the structural meaning of the mimicry between A chain loop antigenic determinants? This observation and that of the

dominance of the A chain loop determinant over the non-loop determinants, as summarized in Table 5, were the impetus for building and studying models of the ungulate insulins. The results of these studies suggest how the conformation of an antigenic determinant of insulin might be influenced by interaction of the antigen with H-2 gene products as well as by the structure of adjacent portions of the insulin molecule.

Table 5. H-2 immune response phenotypes to antigenic determinants of insulin

H-2 genotype	Immunologically-defined antigenic determinant	Immune response phenotype
d	A loop	Cross reaction between A9 Ser-Gly, immunodominant
	Non-loop	Immunorecessive
b	A loop	Responds to A9 Ser>>Gly, A9 Gly primes for A9 Ser>Gly
	Non-loop	No response
k	A loop	Responds to A9 Gly>>Ser, A9 Ser primes for A9 Gly>Ser
	Non-loop	No response

3. UNGULATE INSULINS HAVE A SIMILAR OVERALL CONFORMATION

Differences have been detected between several crystal forms or within one crystal, when the asymmetric unit contains more than one molecule. In crystals of pork insulin (Adams et al., 1969) there are two molecules in the asymmetric unit which are related by a non-cyrstallographic two-fold axis. Hence, the conformations of the two molecules are similar but not identical. The comparison of the two molecules has been reported in fine detail (Dodson et al., 1979) and some differences were observed due to packing of the crystal, most of them in regions that are involved in intermolecular contacts. Some small conformational differences were detected within the antigenic determinant; in particular, the A chain loop A6-A11 in molecule one

is a fairly good alpha helix, whereas in molecule two there is a slight distortion of this helix, so that part of this segment assumes a conformation approximately that of a π-helix. However, in spite of these differences, the intra-atomic contacts within the loop (which are described in detail later), are conserved in both molecules. The overall conformations of both molecules are very similar: The mean value of displacement between them in the asymmetric unit for backbone atoms is 0.11 Å (Dodson et al., 1979) and for the whole molecule as computed using the best molecular fit program it is 1.2 Å.

Three different crystal forms of pork insulin have been determined: the 2-Zn rhombohedral (Adams et al., 1969; Dodson et al., 1979), the 4-Zn rhombohedral (Bentley et al., 1976) and the Zn-free cubic space groups (Dodson et al., 1978). Analysis of the different crystal organizations shows that the conformation of the insulin molecule is grossly indifferent to the crystallization conditions, and only strong packing forces (i.e., the contact between Zn and part of the molecule) have some effect on the structure. This is especially true for the A loop component of the antigenic determinants (Dodson et al., 1979).

Recently, the structure of beef insulin has been determined to a fairly high resolution (2.3 Å), using the molecular replacement method (Blundell, private communication). This structure is now refined to an R factor of 23%, and a difference electron density map between it and pork insulin shows that the conformations of both molecules are almost identical.

The above-mentioned points, together with the immunological observations that antibodies to one ungulate insulin recognize also the other ungulate insulins (Keck, 1975; Barcinki and Rosenthal, 1977) and that all the ungulate insulins bind to the same receptor site on target cells of the hormone, led us to assume that the conformations of the beef and sheep insulins are basically the same as that of pork insulin. Therefore, the main differences are in the side chains of the substituted amino acids.

4. PROCEDURE FOR MODEL BUILDING

The 2-Zn pork insulin coordinates, determined from a highly refined model based on 1.5 Å resolution crystallographic data (Dodson et al., 1979), were kindly supplied by Dr. G.G. Dodson, Department of Chemistry, York University, England).

Since all the insulin molecules investigated in this study have the same number of amino acids, it was possible to build models for the different ungulate insulins without changing the main chain conformation. A substitution of a long side-chain by a shorter one (Ala instead of Val for A8 and Asp instead of Glu in A4) was performed by removing the extra atoms. However, when a short side chain replaced a longer one, the additional atoms were incorporated so that no short contacts were allowed. If it was possible, we preferred to build the so-called "standard conformation."

The details of model building are: 1) Residue B3 in mouse is Lys and in pork, beef and sheep insulins it is Asn. This Lys was built as if it had a fully extended conformation. 2) Pro B9 was easily inserted into the mouse insulin, since the Phy angle of the corresponding Ser in the ungulate insulin is 67.4 deg which is within the limits of a standard proline. 3) Sheep insulin has a glycine in position A9, instead of the serine present in pork, beef and mouse insulins. Although glycine may assume higher flexibility than serine we did not change its conformation. The asymmetric unit contains two molecules of pork insulin related by a non-crystallographic two-fold axis; the BMF (best molecular fit) program was used to compare between them (Nyburg, 1974; Nyburg and Sussman, 1979). This program was also used for insertion of long side chains. The exposed surfaces of components of the determinant were computed using a program written by F.M. Richards and T. Richmond (1978).

5. ANALYSIS OF THE STRUCTURE AND FLEXIBILITY OF THE SUBSTITUTED PORTIONS OF UNGULATE INSULIN

As mentioned above, residues A4, A8-A10, B3, B9, B29 and B30 are hypervariable amino acids in the insulin molecule. All of them are located on the surface of the molecule. Except for B9, all of them are in a fairly close proximity to each other (see Fig. 1). B9 is located at such a distance (about 11.0 Å from the closest determinant amino acid), that it is very unlikely that it forms part of the antigenic determinants related to the other substitutions.

The maximum distance between the atoms of residues A4, A8-10 and B3 is 21.7 Å, and the maximum displacements along the cartesian axes are Δx = 19.6 Å, Δy = 15 Å, Δz = 9.9 Å. If we include in the

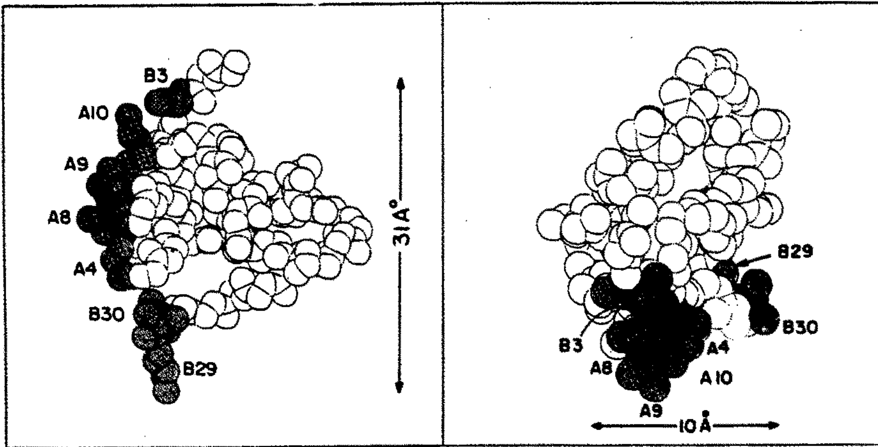

Fig. 1. Van der Waals spheres (radius 1.4 Å) of the backbone atoms of pork insulin, and of the side chains of residues A4, A8-A10, B3, B29, B30, which are shaded. The views looking down the x and y axes are drawn on the right and left sides of the figure, respectively. The coordinates for this figure as well as for Fig. 2, were kindly supplied by G. Dodson.

determinant residues B29 and B30, the maximum distance becomes 32.6 Å, and the displacements along the axes are 31.6 Å, 21.1 Å and 9.9 Å.

The accessible surface of A4, A8-A10 and B3 is 114 $Å^2$. B29 and B30 are much more exposed; their accessible surface areas are 45.1 Å and 40.0 Å, respectively. Can an area of this size be accommodated within a single combining site of a T lymphocyte receptor? The antigen receptor of T lymphocyte has not been characterized chemically. However, immunologic studies using antibodies to receptor idiotypes suggest that the combining site of T lymphocytes is similar to that of antibody molecule binding to the same antigenic determinant (Binz and Wigzell, 1975; Eichman and Rajewsky, 1975). The combining sites of antibodies for haptens have been analyzed crystallographically (Segal et al., 1974; Amzel et al., 1974; Ely et al., 1973; Schiffer et al., 1973), and their dimensions were found to be about 10 Å deep and 15 Å wide, hence we can safely include A4, A8-A10 and B3 within the boundary of an antigenic determinant of insulin that is recognized by a single combining site. However, haptens account for only a part of the available surface of the hypervariable region of antibodies. These regions are usually built as clefts with dimensions of about 30×40 $Å^2$ (Givol, 1979; Davies 1975). Therefore, B29 and B30 might be accommodated within the same binding sites,

Although the side chain of B29 is probably not involved in recognition, since its chemical modification by covalent binding to rhodamine does not alter the immunological activity of the insulin molecule (Talmon, unpublished results). Hence, one antigenic determinant of insulin could consist of 6 residues (A4, A8-A10, B3, B30), in good agreement with the average number of residues found for the myoglobin (Atassi, 1979a) and lysozyme antigenic determinants (Atassi 1979b).

The B (thermal) factor reflects, to some extent, the flexibility of a molecular structure. Therefore, we compared the individual thermal factors of atoms in the substituted portions to those in the reminder of the molecule to elucidate the relative flexibility of the conformation of the antigenic determinant. These values are summarized in Table 6.

Table 6. Analysis of the B (thermal) factors for atoms in the asymmetric unit of insulin

(A) The whole molecule

B value	No. of atoms in molecule 1	No. of atoms in molecule 2
Less than 20	267	240
Less than 30	355	344
Bigger than 40	28	39

(B) Main chain only

B value	No. of atoms in the two molecules
Less than 20	314
Between 20 and 30	73

From this table it is clear that in the region of the antigenic determinant most of the main chain atoms have B values less than or equal to 20. Exceptions are the carbonyl group of Ser A8 of molecule two (B(C) = 37, B(O) = 25) and the carbonyl and Cα of Asn in the same molecule (B(Cα) = 45, B(O) = 30, B(C) = 29). The atoms of the side

chains have values between 20 and 30, except for the side chain atoms (Nδ, Oβ, Cβ) of B3 Asn in molecule 2 that has a value higher than 40. B29 and B30 in both molecules are clearly more flexible than most of the molecule. Their B values average around 35 and for extreme cases (such as Nξ of lysine) may reach 90. Hence, on the whole, the region of the antigenic determinant has a well-defined conformation and is probably not more nor less flexible than the other parts of the molecule, although it is located on the surface of the molecule.

6. FINE STRUCTURAL ANALYSIS IN RELATION TO IMMUNOLOGIC MIMICRY AND DOMINANCE

To gain some insight into the mimicry of serine (A9) by its substituted glycine and the dominance of the loop over the non-loop substitutions (Table 5), we focused our attention on the possible van der Waals contacts and H-bonds that are made by components of the antigenic determinant. A list of such contacts for the different insulins is given in Table 7. It is of particular importance to note that (a) the side chain of Lys B29 does not interact with any other part of the antigenic determinant. It has been observed that at least in molecule one it exists in two conformations; (b) B30, which is the c-terminal amino acid, interacts only with A1 and is partially disordered in the crystal state. In molecule 2 it has two conformations;

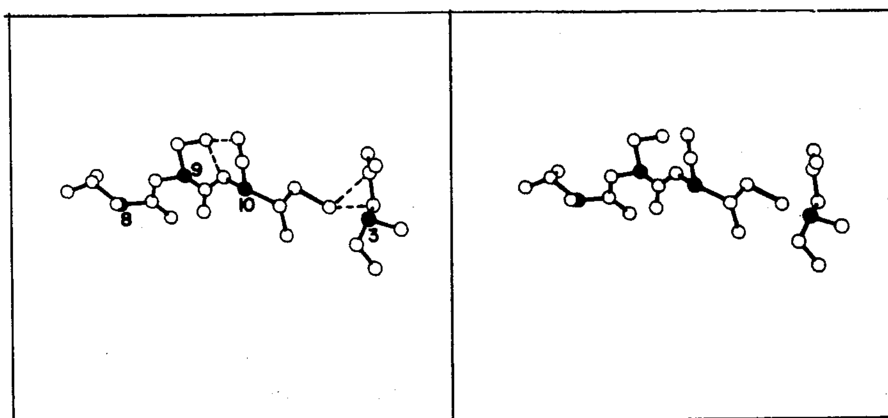

Fig. 2. The conformation of A8-A10 and B3 in pork insulin molecule (Dodson et al., 1979). The alpha carbons are black. Possible hydrogen bonds and van der Waals contacts are shown in broken lines.

Table 7. Intermolecular contacts

Atom	To atom	Type	Distance (Å)
Thr A8 Oγ1	Thr A8 N	Pork, mouse	3.0
	Ser A9 N	Pork, mouse	3.1
Thr A8 Cγ2	Glu A4 O	Pork	3.4
	Asp A4 O	Mouse	3.4
Ser A9 Cβ	Gln A5 O	Pork, mouse, beef	3.3
Ser A9 Oγ	Ile, Val A10 N	Pork, mouse, beef	2.9
Ser A9 Oγ	Ile, Val O	Pork, mouse, beef	3.0
Ile A10 Cδ1	Asn B3 Nδ2	Pork	3.3
	Asn B3 Cβ	Pork	3.6
	Lys B3 Cγ	Mouse	3.3
	Lys B3 Cβ	Mouse	3.6

(c) residue A9 interacts with A4 only through its backbone; (d) Ile A10 makes hydrophobic contacts with Cβ and Cγ of residue B3, no matter whether it is lysine or asparagine (Fig. 2). However, when Ile (A10) of mouse and pork insulins is replaced by Val (as in beef and sheep insulins) these interactions are missing. It is clear, therefore, that the substitution of isoleucine by valine at A10 could be more consequential than the substitution of a lysine by asparagine at B3. It is of interest that the location and the conformation of B3 is well preserved in the two molecules of the 2-Zn insulin dimer as well as in one molecule of the 4-Zn insulin dimer (Dodson et al., 1979; Bently et al., 1976). In the 4-Zn crystal form, B3 is very close to the Zn binding site, and the location and conformation of one molecule are different from the

above-mentioned three. Unfortunately, the coordinates of the 4-Zn crystal form have not yet been refined to the same level as those of the 2-ZN form, and therefore a detailed comparison is still not possible; (e) Oγ of Ser A9, as well as Oγ of Thr A8 are located so that they can make H-bonds with a carbonyl and an NH group of the neighboring main chain (Fig. 2). When serine is substituted by a glycine, the vacant place of the Cβ-OH may be occupied by a water molecule. This water molecule can sit so that it is able to make an H-bond with the carbonyl and amino groups in the same manner that the OH of the serine does, and therefore can mimic the serine side-chain.

7. DISCUSSION OF THE RELATIONSHIP BETWEEN MOLECULAR STRUCTURE AND SELECTION OF ANTIGENIC DETERMINANTS BY Ir GENE PRODUCTS

Based on cross-reactivities between substituted insulins, we identified the antigenic determinants of ungulate insulins that are recognized by mouse T lymphocytes under the direction of Ir genes. Our conclusions (Table 5) were derived from the experimental observations and from the assumption that self-antigenic determinants present on mouse insulin were probably not recognized in the immune response. We found that an A chain loop determinant was recognized by mice of the $H\text{-}2^d$, $H\text{-}2^b$ and $H\text{-}2^k$ genotypes. A non-loop determinant was recognized only by $H\text{-}2^d$ mice and this determinant was immunologically recessive to the loop determinant. $H\text{-}2^b$ and $H\text{-}2^k$ mice reacted to different variants of the loop determinant; $H\text{-}2^b$ preferring to see beef (A9 Ser) and $H\text{-}2^k$ preferring to see sheep (A9 Gly) insulins. These variants of the loop determinant could mimic each other, since immunization of $H\text{-}2^b$ or $H\text{-}2^k$ mice with the "forbidden" variant primed them for a secondary immune response to the "permitted" or preferred variant. $H\text{-}2^d$ mice did not distinguish between A9 Ser and A9 Gly Cohen and Talmon, 1980).

How might we interpret the behavior of the immune system in the light of our analysis based on crystallographic studies of pork insulin and model building of the structure of the substituted regions of the ungulate insulins? Despite the fact that the conformations of any molecule in the crystal state may not represent the range of conformations taken by the molecule in vivo, we believe that a comparison

between immunological and crystallographical analyses is useful. Such comparison may challenge or extend interpretations of the biological phenomena and raise new questions for experimental investigation.

Although only the substitutions in the loop (A8-A10) are sequentially related, the three-dimensional structure of insulin places the other substitutions (A4, B3 and B30) in contiguity with A8-A10 across the surface of the molecule (Fig. 1). Only position B9, which differs in mouse insulin I but not in II, is outside of this hypervariable patch. Positions A6 through A11 are a loop, since they are linked by an s-s bond, but this loop does not appear as a conformational entity on the surface of the molecule. Thus, the contribution of A8, A9, A10 to the surface conformation is not as a loop but rather as a part of the continuum or patch of substituted residues. Therefore, it would seem to be structurally inexact to talk of a "loop determinant."

According to its surface area, the bulk of the hypervariable patch of 6 amino acid residues could be accommodated within a combining site of a single antibody or, presumably, of a T lymphocyte. This raises the possibility that the entire hypervariable patch (A4, A8, A9, A10, B3, B30) serves as a single antigenic determinant, of which the immunologically critical portion is A8-A9-A10. If this is the case, then the so-called "non-loop" determinant recognized in pork insulin by H-2^d mice might reside on an unsubstituted part of the molecule and constitute a self-antigenic determinant. In this light, we could understand the immunologically recessive nature of a self-determinant when it is combined with a foreign determinant defined by substitutions at A8 and A10 in sheep or beef insulins. In fact, we have observed (Cohen et al., 1979) that pork insulin and not beef insulin sometimes can induce an autoimmune response to mouse insulin.

If the so-called "non-loop" determinant is nevertheless part of the hypervariable patch, the contact between A10 and B3 could explain the immunodominant inlfuence of a residue within the "loop" on the conformation of a "non-loop" side chain. Whether or not the "non-loop" determinant is a self or a substituted structure, it seems that its conformation remains intact in the isolated reduced B chain. It has been shown that pork insulin and its isolated B chain cross react for T lymphocytes of H-2^d mice (Rosenwasser et al., 1979).

Our model building suggested a novel hypothesis to explain the immunologic mimicry between A9 Gly (sheep) and A9 Ser (beef) variants (Table 5). It appears that the OH of A9 Ser can make good H-bonds with carbonyl and NH groups of A10 (Fig. 2). These stabilizing H-bonds would be available to the OH of a water molecule that could fit in the space provided by the A9 Gly substitution. Therefore, the contour of A9 Gly might look similar to that of A9 Ser under particular conditions of hydration; in this way, A9 Gly could mimic A9 Ser.

We do not know how the products of Ir genes influence the selection of antigenic determinants on an immunogenic molecule; however, it is conceivable that they control the orientation of the immunogen during its presentation by macrophages to the receptors of T lymphocytes (Cohen et al., 1979; Cohen and Talmon, 1980). Our hydration hypothesis, derived from model building, proposes that the arrangement of water molecules over the surface of an immunogen, by modifying the contour of the molecule, could influence the affinity of binding of antigenic determinants to particular lymphocyte receptors. This would, in turn, influence the identity or behavior of competing clones of lymphocytes activated by contact with the immunogen (Grossman and Cohen, in press). Ir gene products thus might regulate the process of selection of clones of lymphocytes by physically associating with the immunogen so as to control its orientation and state of hydration during presentation to lymphocytes.

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