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INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

LAKES OF LIQUID SULPHUR

RECOMBINANT DNA
product review

THIS WEEK

Setting the baseline

The greenhouse effect, its existence, extent and implications, has been the subject of hundreds of model and theoretical studies. Yet the physical basis of the effect — the infrared radiation energy trapped by atmospheric gases and clouds — has not previously been measured from observational data. Raval and Ramanathan (page 758) have now filled that gap, presenting an effective method for directly monitoring future changes in the greenhouse effect. See also News and Views, page 736.

Channel vision

The light-sensitivity of vertebrate photoreceptor cells depends on the closure of a cation channel which is gated directly by cyclic GMP, the internal messenger of visual transduction. Now, the cloning and sequencing of the complementary DNA for this channel from bovine retinal rod photoreceptors shows that it could belong to a new family of ligand-gated ion channels. Page 762.

Regal matters

Worker honeybees efficiently destroy worker-laid male eggs if a queen is present. This 'worker policing' ensures that the colony's male reproductives are sons of the queen, pages 796 and 741.

Positional cues

The *Hox-5* homoeogenes are activated in the developing limb bud of the chick in a temporal order that corresponds to their spatial ordering on the chromosome, and in a series of partially overlapping spatial domains such that the region in which all five genes are expressed coincides with the zone of polarizing activity that determines the antero–posterior limb axis. Pages 767 and 734.

Insulin mechanism

Changes in our understanding of how insulin regulates glucose uptake are likely to follow the discovery that the endothelial cells that line the capillaries of the insulin-regulated tissues, muscle and fat express high levels of the insulin-regulated glucose transporter. Page 798.

Beneath the rings

Infrared imaging has revealed several new features in the atmosphere of Saturn — including a hotspot at the north pole and an asymmetric structure at the equator. Page 777.

Yeast shows the way

The processes of differentiation by induction of different gene regulatory proteins under the influence of cell–cell signals, and of the establishment of stem cell lineages, fundamental to metazoan development, can be illustrated in the single-celled yeast. Recent work illuminates the molecular basis of some of these processes. Review Article, page 749. See also page 830.

Hairpin corner

Oligonucleotide encoding telomeric DNA sequences form stable dimers in solution, possibly by the formation of hydrogen bonds between two intramolecular hairpin loops to form antiparallel quadruplexes containing cylic guanine base tetrads. Pages 825 and 737.

Alvin's finds

Samples of sediment taken by the research submersible *Alvin* at the Guaymas Basin hot vents (Gulf of California), contain a group of methanogenic archaebacteria growing, quite unexpectedly, at a temperature of at least 110°C (page 833). From the same area, dense layers of large filamentous sulphur-oxidizing bacteria of the genus *Beggiatoa* have been found at a depth of 2010 m (page 834).

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Conformations of immunoglobulin hypervariable regions

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On the basis of comparative studies of known antibody structures and sequences it has been argued that there is a small repertoire of main-chain conformations for at least five of the six hypervariable regions of antibodies, and that the particular conformation adopted is determined by a few key conserved residues. These hypotheses are now supported by reasonably successful predictions of the structures of most hypervariable regions of various antibodies, as revealed by comparison with their subsequently determined structures.

THE relationships between the amino-acid sequences of immunoglobulins and the structures of their antigen-binding sites are important for understanding the molecular mechanisms of the generation and maturation of the immune response and for designing engineered antibodies. Antigen-binding sites are formed by six loops of polypeptide, the hypervariable regions; three from the variable domain of the light chain (VL) and three from the variable domain of the heavy chain (VH), denoted L1, L2, L3, and H1, H2, H3, respectively (Fig. 1a). Within the domains, the loops are connected to a β-sheet framework whose structure is conserved[1,2]. The specificity and affinity of the binding sites are governed by the structures of the six hypervariable regions[3,4].

Two models can be proposed for the relationship between the amino-acid sequence and structure of the binding-site loops. In one model, different sequences produce different conformations for both the main chain and side chains of the loops. Because hypervariable regions have different sequences in different antibodies, this model implies that each region adopts a different conformation in different antibodies. In the other model, antibodies have only a few main-chain conformations or 'canonical structures' for each hypervariable region. Most sequence variations would only modify the surface provided by the side chains on a canonical main-chain structure. Sequence changes at a few specific sets of positions would switch the main chain to a different canonical conformation.

Canonical structure model

Experimental evidence indicates that the canonical structure model describes the relationship between amino-acid sequence and structure for at least five of the six hypervariable regions[5-9]. Kabat *et al.*[5] found conserved residues at sites within certain sets of hypervariable regions and suggested that they had a structural role. Padlan and Davies[6], and more recently de la Paz *et al.*[7], showed that some of the hypervariable regions in the immunoglobulins of known structure have the same main-chain conformation in spite of several differences in sequence.

Chothia and Lesk[8] identified the residues that through packing, hydrogen bonding, or the ability to assume unusual values of the torsion angles ϕ, ψ or ω, are primarily responsible for the main-chain conformations of the hypervariable regions in the structures then known—the Fab fragments of NEW (ref. 10), McPC603 (ref. 11), KOL (ref. 12) and J539 (ref. 13) and the VL domains of REI (ref. 14) and RHE (ref. 15). The conformations are determined by the interactions of a few residues at specific sites in the hypervariable regions and, for certain loops, in the framework regions. Hypervariable regions that have the same conformations in different immunoglobulins have the same or very similar residues at these sites (Fig. 1 and Table 1). Examination of the amino-acid sequence of the antibody D1.3 showed that its hypervariable regions are the same size as those in known structures and contain the same or similar residues at the sites responsible for known conformations[9]. On the basis of these observations the atomic structure of the VL–VH dimer of D1.3 was predicted before its experimental determination. Comparison of this predicted structure with the preliminary crystal structure showed that the conformations of four of the hypervariable regions had been predicted correctly; the conformation of L3 was significantly different from that predicted, and

TABLE 1   Sequences and conformations of Vκ and VH hypervariable regions of known structure

L1 Regions†

Canonical Structure	Protein	26	27	28	29*	30	31	a	b	c	d	e	f	32	2*	25*	33*	71*
1	J539	S	S	S	V	S	—	—	—	—	—	—	—	S	I	A	L	Y
	HyHEL-5	S	S	S	V	N	—	—	—	—	—	—	—	Y	I	A	M	Y
	NQ10	S	S	S	V	R	—	—	—	—	—	—	—	Y	I	A	M	Y
2	REI	S	Q	D	I	I	K	—	—	—	—	—	—	V	I	A	L	Y
	D1.3	S	G	N	I	H	N	—	—	—	—	—	—	Y	I	A	L	Y
	HyHEL-10	S	Q	S	I	G	N	—	—	—	—	—	—	N	I	A	L	F
	NC41	S	Q	D	V	S	T	—	—	—	—	—	—	A	I	A	L	Y
3	McPC603	S	E	S	L	L	N	S	G	N	E	K	N	F	I	S	L	F
4	4-4-20	S	Q	S	L	V	H	S	—	N	G	N	T	Y	V	S	L	F

Total no. of sequences known for L1 regions: human, 95; mouse, 299.

Canonical structure	1	2	3	4
Human sequences that fit (%)	—	60	5	5
Mouse sequences that fit (%)	15	25	20	10

L2 Regions

Canonical Structure	Protein	50	51	52	48*	64*
1	REI	E	A	S	I	G
	McPC603	G	A	S	I	G
	J539	E	I	S	I	G
	D1.3	Y	T	T	I	G
	HyHEL-5	D	T	S	I	G
	HyHEL-10	Y	A	S	I	G
	NC41	W	A	S	I	G
	NQ10	D	T	S	I	G
	4-4-20	K	V	S	I	G

Total no. of sequences known for L2 regions: human, 69; mouse, 183.

Canonical structure	1
Human sequences that fit (%)	95
Mouse sequences that fit (%)	95

L3 Regions

Canonical Structure	Protein	91	92	93	94*	95	96	90*
1	REI	Y	Q	S	L	P	Y	Q
	McPC603	D	H	S	Y	P	L	N
	D1.3	F	W	S	T	P	R	H
	HyHEL-10	S	N	S	W	P	Y	Q
	NC41	H	Y	S	P	P	W	Q
	4-4-20	S	T	H	V	P	W	Q
	NQ10	W	S	S	N	P	L	Q
2	J539	W	T	Y	P	L	I	Q
3	HyHEL-5	W	G	R	N	P	—	Q

Total no. of sequences known for L3 regions: human, 52; mouse, 152.

Canonical structure	1	2	3
Human sequences that fit (%)	90	—	2
Mouse sequences that fit (%)	80	10	1

H1 had a very different fold from that predicted[9]. (We report below that the refined conformation of D1.3 corresponds more closely to the predicted structure.)

An examination of the library of the known immunoglobulin sequences shows that many immunoglobulins have hypervariable regions that are the same size as those in the known structures and contain the same or closely related residues at the sites responsible for the known conformations[8]. These observations indicate that for at least five of the hypervariable regions there is only a small repertoire of canonical main-chain conformations and that the conformation actually present can often be predicted from the sequence by the presence of specific residues.

The accuracy of the canonical structure model for immunoglobulin binding sites depends on (1) the correct determination of the sets of residues responsible for the observed conformations and (2) changes in the identity of residues at other sites not significantly affecting the conformations of the canonical structures. The model can be tested, refined and extended by using it to predict the atomic structures of binding sites in immunoglobulins before their structures have been determined by X-ray crystallography.

We have now tested the canonical structure model by using it to predict the structures of four immunoglobulins before their structures had been experimentally determined. These immunoglobulins are HyHEL-5 (ref. 16), HyHEL-10 (ref. 17), NC41 (ref. 18) and NQ10 (S.S., P.M.A. and R.J.P., manuscript in preparation). The analysis of the amino-acid sequences of these immunoglobulins indicated that 19 of their 24 hypervariable regions should have conformations close to known canoni-

H1 Regions‡

Canonical Structure	Protein	26	27	28	29	30	31	32	34	94
		*	*		*				*	*
1	McPC603	G	F	T	F	S	D	F	M	R
	KOL	G	F	I	F	S	S	Y	M	R
	J539	G	F	D	F	S	K	Y	M	R
	D1.3	G	F	S	L	T	G	Y	V	R
	HyHEL-5	G	Y	T	F	S	D	Y	I	R
	NC41	G	Y	T	F	T	N	Y	M	R
	NQ10	G	F	T	F	S	S	F	M	R
	4-4-20	G	F	T	F	S	D	Y	M	G
1'	NEW	G	S	T	F	S	N	D	Y	R
	HyHEL-10	G	D	S	I	T	D	D	W	N

Total no. of sequences known for H1 regions: human, 50; mouse, 321.

Canonical structure	1
Human sequences that fit (%)	50
Mouse sequences that fit (%)	80

H2 Regions§

Canonical Structure	Protein	52a	b	c	53	54	55	71
							*	
1	NEW	—	—	—	Y	H	G	
	D1.3				G	D	G	
	HyHEL-10	—	—	—	Y	S	G	
		*					(•)	*
2	HyHEL-5	P	—	—	G	S	G	A
	NC41	T	—	—	N	T	G	L
						(+)		*
3	KOL	D	—	—	D	G	S	R
	J539	P	—	—	D	S	G	R
	NQ10	S	—	—	G	S	S	R
						(•)	*	*
4	McPC603	N	K	G	N	K	Y	R
	4-4-20	N	K	P	Y	N	Y	R

Total no. of sequences known for H2 regions: human, 54; mouse, 248.

Canonical structure	1	2	3	4
Human sequences that fit (%)	15	1	40	15
Mouse sequences that fit (%)	15	40	5	20

The residues listed here (single-letter code) are those that form the hypervariable regions and those in the framework regions that are important for the observed conformations of these regions[6]. The hypervariable regions are taken as those outside the framework β-sheet[6]. Except for H2, they are similar to, but not identical with the regions that show high sequence variations and which Kabat et al.[26] use to define hypervariable regions. The sequences are grouped so that those that have the same main-chain conformation, or canonical structure, are adjacent. The canonical structure numbers used below refer to the conformations shown in Fig. 1. The residues in the hypervariable and framework regions that are mainly responsible for these conformations[6] are indicated by an asterisk. The classification and sequence requirements of the H2 conformations have been revised in the light of work described here and elsewhere[28]. For each hypervariable region the number of human and mouse sequences listed by Kabat et al.[26] are given. We also give the percentage of these sequences that are the same size as the known canonical structures and have the same residues at the positions marked by an asterisk.

‖ Canonical structure 4 is illustrated in Fig. 4. Although the size of the known L1 structures varies between 6 and 13 residues, they have closely related folds with residues 26-19 and 32 packed against the framework in the same conformation[6]. The remaining residues form a turn or loop on the surface (Figs 1 and 4). The ends of the long loops have some flexibility. There are another 25% of the human sequences and 20% of the mouse sequences that have one more residue than structure 2, or one fewer than structure 4, and whose sequences satisfy the requirements listed above. It is expected that these differ only in the conformations of the tips of the surface loops.

‡ The H1 hypervariable regions with canonical structure 1 have very similar conformations: the r.m.s. differences in the coordinates of their main-chain atoms are 0.3-0.8 Å. The H1 regions in NEW and HyHEL-10 only partly satisfy the sequence requirements for structure 1 and have a distorted version of its conformation.

§ The H2 region here comprises residues 52a-55. The region with high sequence variation is 50-65 (ref. 26). In the known structures the main-chain conformation of 50-52 and 56-63 do not differ significantly[6] (Fig. 1b). (•), The residues at positions 55 or 54 in the canonical structures 2, 3 and 4 have residues with positive values for φ and ψ, and usually, but not in all cases, Gly, Asn or Asp is found at these sites. For a sequence to match that of canonical structure 2, 3 or 4 the presence of these residues at sites 54 or 55 is required.

cal structures. We then compared the predicted structures of these hypervariable regions with the subsequently determined structures. Another immunoglobulin structure, 4-4-20 (ref. 19) has recently been reported. We did not have the opportunity to predict the structure of 4-4-20 before its experimental determination, and we discuss here only how its hypervariable regions have the conformations expected from the known canonical structures. Also, we report that the refined conformation of D1.3 (ref. 20) corresponds more closely to the predicted structure.

Model building procedure

The main-chain conformations of the hypervariable regions in the Vκ and VH domains of known structure are shown in Fig. 1. The residues responsible for these conformations are listed in Table 1. Each hypervariable region in the immunoglobulins of unknown structure was examined to determine (1) whether it has the same size as any homologous hypervariable region of known structure and (2) whether its sequence contains the set of residues responsible for a known conformation. Except for L3 in HyHEL-5, all the light-chain regions correspond to a known canonical structure, as do all the H1 regions and the H2 region in HyHEL-10 (Table 1). The conformation predicted for the H2 regions in NC41 and HyHEL-5 was based on the analysis of the H2 region in the preliminary structure of J539 (ref. 8). In all three of these antibodies the H2 region is a four-residue turn with Gly at the fourth position and the predicted conformation is that almost always found for such turns[21]. (Below we present a more accurate analysis of H2 regions.) For H3 regions in HyHEL-5, HyHEL-10, NC41 and NQ10, no prediction of

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