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A Mouse Ig κ Domain of Very Unusual Framework Structure Loses Function when Converted to the Consensus*

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Antibody gene sequences, particularly those of κ light chains, are very well conserved in the framework region, and the variability is concentrated in the complementarity-determining regions (CDR). We now found that the murine antibody 93-6 (Djavadi-Ohaniance, L., Friguet, B., and Goldberg, M. (1984) *Biochemistry* 23, 97–104) whose F_{ab} fragment binds the β-subunit of *Escherichia coli* tryptophan synthase with high affinity (K_d of $6.7 \cdot 10^{-9}$ M) has a highly unusual κ light chain framework, which is crucial for the function of this antibody. It carries an insertion of 8 amino acids in a conserved framework loop that faces the antigen, and its framework region 2 (FR2) which precedes CDR2 is shortened by one amino acid, normally leucine and part of an absolutely conserved β-bulge preceding CDR2. Removal of the insertion to restore the consensus sequence reduced the binding affinity of 93-6 by a factor 3, while insertion of the missing leucine into FR2 completely abolished binding.

Antibody combining sites, arising by a combinatorial recombination of a limited number of gene segments, can be complementary to almost any antigenic structure. The basic shape of the variable domains V_H and V_L is a β-sheet sandwich, and the folding pattern of the strands is termed the Greek key motif. The variability is concentrated in six surface-exposed loops, the complementarity-determining regions (CDRs).[1] Length variations in these loops are important for the shape of the combining site. The length diversity of the first two CDRs is given by the diversity of the V genes. In contrast, the length variation of the CDR3 of the heavy chain involves the junctional diversity of various V, D, and J segments as well as noncoded bases. The framework regions are highly conserved between and within variable domain classes and essentially no insertions and deletions are observed.

This knowledge is based on about 30 crystal structures of antibody variable domains and about 10^3 sequences, mostly of human and murine origin (Kabat *et al.*, 1991; Rees *et al.*, 1994). For man, probably all V_H genes (Chothia *et al.*, 1992; Tomlinson *et al.*, 1992), most V_κ genes (Cox *et al.*, 1994; Weichhold *et al.*, 1993; Lautner-Rieske *et al.*, 1993) and many V_λ genes (Williams and Winter, 1993) are known. In mouse, in contrast, our knowledge is based mostly on sequencing of many individual monoclonal antibodies. It is thus currently unclear what percentage of mouse V_H and V_κ genes are known. In mouse, there are only three V_λ genes (Selsing *et al.*, 1982; Sanchez *et al.*, 1990), and they are rarely used. From the known sequences, and especially from the known structures, a picture emerges with V_κ genes particularly well conserved (Kabat *et al.*, 1991; Rees *et al.*, 1994), limiting the structural variations to the CDR regions and only displaying somatic point mutations in a series of very similar frameworks.

In this study, we report a mouse κ chain, found in a functional antibody, which is different from any other in the data base in two absolutely conserved features. We also show that it loses function when converted back to the consensus.

MATERIALS AND METHODS

Cloning—Hybridoma 93-6 (IgG1) (Djavadi-Ohaniance *et al.*, 1994) was grown in RPMI 1640 medium, supplemented with heat-inactivated horse serum, pyruvate, penicillin, and streptomycin. 270 μg of mRNA were isolated from 5×10^7 cells using the mRNA isolation kit from Pharmacia Biotech Inc. 6.7 μg of mRNA were used as template for cDNA synthesis using the Pharmacia 1st strand cDNA synthesis kit. *Not*I-d$(T)_{18}$ or 3′ primers (specific for C_H1 and C_L) were used as primers. The PCR, carried out as described by Huse *et al.* (1989) using cDNA mRNA hybrids as the template, gave rise to fragments of the expected sizes. V_HC_H1 and V_LC_L genes were cloned into the vector pSL301 (Invitrogen Corp.) as *Xho*I-*Spe*I and *Hin*dIII-*Sac*I fragments and sequenced. In a separate experiment, the primer sets for V_H and V_L from the antibody PCR kit from Pharmacia were used instead, with subsequent assembly to a scFv as described by the manufacturer. The same sequence was obtained as with the Fab primers modified from Huse *et al.* (1989).

Expression of the scFv Fragment—The scFv fragment of the antibody 93-6 was assembled by PCR of the Fab fragment and subcloned into the pIG6 expression vector using primers SC-1, -2, -3, and -4 (Ge *et al.*, 1995). The extra loop (EL) in FR3 was converted to the consensus sequence (QNRSPFGNQLN → GTD) by site-directed mutagenesis using a Bio-Rad kit, whereby a *Bsp*EI site was destroyed. The "missing" leucine (Leu-47) was added (L^+, EL^-L^+) by site-directed mutagenesis with an oligonucleotide containing a *Hin*dIII site. The wild type as well as the mutants (EL^-, L^+, EL^-L^+) were confirmed by DNA sequencing. The clones of pIG6-scFv (93-6) wild type, EL^-, L^+, and EL^-L^+ in *Escherichia coli* JM83 were grown in LB medium containing streptomycin (25 μg/ml) and ampicillin (100 μg/ml). The fresh cultures of $OD_{550\,nm} \sim 0.4–0.6$ were induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside and shaken at room temperature for 3 h. The periplasmic fractions were obtained and scFv fragments were purified by immobilized metal ion chromatography as described elsewhere (Ge *et al.*, 1995; Lindner *et al.*, 1992).

Cloning and Expression of Tryptophan Synthase β-Subunit—The

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) Z48767 and Z48768.

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[1] The abbreviations used are: CDR, complementarity-determining region; PCR, polymerase chain reaction; EL, extra loop; ELISA, enzyme-linked immunosorbent assay; FR, framework region; scFv, single chain Fv fragment.

tryptophan synthase β-subunit was PCR amplified from the genome of *E. coli* with one primer encoding an N-terminal addition of 6 histidines. This is structurally far removed from the known epitope for the antibody 93-6 (Friguet *et al.*, 1989, 1993). The protein was purified by immobilized metal ion chromatography on Ni-nitrilotriacetic acid (Lindner *et al.*, 1992).

ELISA—The antigen was coated at 2 μg/ml on special luminescence ELISA plates (Dynatech), and the wells were blocked with milk powder. The scFv fragments were detected with the anti-*myc*-tag antibody 9E10 (Munro and Pelham, 1986) and a goat anti-mouse alkaline phosphatase conjugate. The phosphatase activity was detected with the ELISA-light kit using disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro)-tricyclo[3.3.1.1^{3,7}]decan}-4-yl)-phenyl phosphate (Tropix) in a ML3000 luminometer (Dynatech).

Determination of Binding Constants—In the measurements of the binding constants, $3 \cdot 10^{-8}$ M to 10^{-7} M scFv fragment was incubated with varying amounts of tryptophan synthase β-subunit in 0.9 ml of phosphate-buffered saline containing 1 mg/ml bovine serum albumin at 4 °C for 16 h. Aliquots of 0.2 ml were removed for determining the unbound scFv, and the amount of tryptophan synthase used in coating was first tested to be small enough to not displace the equilibrium (Friguet *et al.*, 1985). Triplicate measurements on two different protein preparations were used for each mutant. The binding constant was obtained by fitting the ELISA signal directly to the law of mass action using the relation $A = A_0 \cdot (1 - [Ab \cdot Ag]/[Ab_{tot}])$, where A and A_0 are the measured absorbance and the value in the absence of soluble antigen, respectively, and

$$[Ab \cdot Ag] = \frac{[Ab_{tot}] + [Ag_{tot}] + K_D}{2} - \sqrt{\left(\frac{[Ab_{tot}] + [Ag_{tot}] + K_D}{2}\right)^2 - [Ag_{tot}] \cdot [Ab_{tot}]}.$$

The total concentrations of antibody and soluble antigen are given by Ab_{tot} and Ag_{tot}, respectively, and K_d is the dissociation constant.

Modeling—Of the V_H sequences in the Protein Data Bank (PDB; Brookhaven National Laboratories, April 1993), those of antibodies 36-71, R19.9, and HyHel5 were most similar to V_H of 93-6 (73–77% identity). The similarity to other antibodies was significantly lower. The 93-6 V_H main chain was modeled on R19.9, except for CDR H2, which was modeled on HyHel5 because this antibody also binds a protein rather than a small ligand. The model of CDR H2 must be considered with caution, since it contains three consecutive glycines that are largely unconstrained by the rest of the molecule. CDR H3 of 93-6 was shorter than that of 19.9 but also contained residues Arg-94 and Asp-101 that form a salt bridge and have a conserved structure in all antibodies that contain them. Only four residues were left in 93-6 to close the loop between Arg-94 and Asp-101, and because the second of these was a glycine, it was considered likely that the loop would form a type II′ turn (Sibanda *et al.*, 1989). As a control, CDR H3 was modeled on CDR H3 of AN02, the only one in PDB to have the same length. The *cis*-Pro in AN02 was excised, and the backbone was converted to a *trans* conformation, then steric clashes were relieved manually and the loop was energy-minimized *in vacuo* without charges and with a planarity constraint on the peptide bonds. The final structure was very similar (main chain root mean square deviation <1 Å) to the one obtained in the other approach. After modeling the main chain of V_H, the side chains were modeled by maximum overlap to 36-71, R19.9, and HyHel5. In cases of discrepancy between the structures, which were few and generally seen in solvent-exposed positions, the structure with the most favorable torsion angles (Ponder and Richards, 1987) was used. The side chains in loop H3 were added after building the V_H-V_L dimer.

V_L was most similar to a mutant of McPC603 containing the CDR1 region of MOPC167 (1IMN) (55% identity) and also had CDR loops of the same length, so that this protein was used as a template. For CDR L1, a certain degree of uncertainty stems from the fact that, although all four CDR L1 loops of this length in PDB (B13I2, 4-4-20, 26-10 and McPC603/MOPC167) form type I + G1-bulge turns, their position relative to the light chain framework is fairly variable and all four have Gly at position 29, while 93-6 has Ser at this position and Gly at position 28. To explore alternate conformations, we extracted all 3:5, 5:5, 5:7, and 7:7 loops from the compilation of Sibanda *et al.* (1989) that contained Gly in the same position as 93-6. Most of these were further reclined than CDR L1 of McPC603/MOPC167; the loop formed by residues 17–23 of staphylococcal nuclease could be overlapped best with the base of CDR L1 and was included into the model as an alternative. As described in the text, two models were generated for the CDR L2 region: one in which the β-bulge was preserved and the following γ-turn was changed to a β-turn, and one where the β-bulge was changed to a β-strand and the framework loop following CDR L2 was shifted to compensate for the residue missing in the core. Although we made conservative assumptions about this shift, the actual structure may undergo more significant alterations since two highly conserved residues in this area are not present in 93-6: Pro-59 is Ser and Gly-64 is Asp (possibly replacing with its side chain a conserved internal water molecule in the back of CDR L2).

For a model of the insertion, we assumed that it would form a β-hairpin with a type I turn because of Pro in the i + 1 and Gly in the i + 4 position (Sibanda *et al.*, 1989). Using the procedure of Jones and Thirup (1986), we found eight loops with good main chain overlap. Because of Gly-69E at the base of the turn (Fig. 1), the loops showed significant variability. The loop formed by residues 28–37 of 3EBX was best compatible with Pro in position i + 1. We positioned the side chains of 93-6 V_L by maximum overlap to McPC603/MOPC167 after fitting in the insertion but prior to modeling CDR L2. Residues without correspondence were given the most favorable torsion angles (Ponder and Richards, 1987) that did not result in steric overlap. After the CDR L2 models were made, both structures were subjected to 500 rounds of steepest descents energy-minimization *in vacuo* without charges and a fixed backbone to relieve close contacts. Finally, the V_L model was complexed to the V_H model by maximum overlap of its main chain atoms at the V_H-V_L interface with V_L of R19.9. Side chains were then added for CDR H3 and the entire model was again subjected to 500 rounds of steepest descents energy minimization *in vacuo* without charges and with a fixed backbone.

The approximate docking orientation was derived from steric considerations, since most relative orientations can be excluded because of significant overlaps. Only an almost perpendicular orientation of the V_H-V_L axis relative to the αββα axis appeared to be possible, in the absence of major structural rearrangements upon complex formation.

RESULTS AND DISCUSSION

The antibody 93-6, directed against the β-subunit of *E. coli* tryptophan synthase, was obtained by hybridoma technology (Djavadi-Ohaniance *et al.*, 1984). We amplified the genes of the Fab fragment by PCR of hybridoma mRNA, using primers hybridizing in the constant domains C_H1 and C_L and primers hybridizing to the 5′ end of the V genes, which were adapted from the literature (Huse *et al.*, 1989). To exclude any PCR artefacts, the mRNA was independently amplified again by PCR with a different set of commercial primers hybridizing to the 5′ and 3′ sequences of the V genes (see "Materials and Methods") (Fig. 1). In both cases, the same antibody sequence was obtained (DNA sequence submitted to EMBL, accession nos. Z48767 and Z48768).

While the heavy chain is a typical member of mouse heavy chain subgroup V(a) (Fig. 1*A*), the κ light chain differs in two important aspects from any other κ chain in the data base. First, it has an insertion of 8 amino acids in FR3 (after residue 69, Kabat numbering) (Kabat *et al.*, 1991), which forms a turn at the antigen binding face of the molecule. Second, the sequence is missing a leucine residue in FR2 that normally gives rise to a β-bulge at the base of CDR2 (Fig. 1*B*).

To determine whether these deviations from the consensus κ sequence are of functional importance, we expressed the protein as a single chain Fv (scFv) fragment in *E. coli* (Glockshuber *et al.*, 1990; Ge *et al.*, 1995) and constructed three mutants: one in which the FR3 insertion is removed by converting the nontypical 11 residue stretch (highlighted in Fig. 1) to the consensus sequence GTD ("extra loop deleted," denoted EL^-), one in which the missing leucine residue is inserted into FR2 ("Leu insert," denoted L^+), and one that contains both mutations (denoted EL^-L^+). The scFv fragments all contain a shortened 3-amino acid long N-terminal FLAG (Knappik and Plückthun, 1994) and a C-terminal *myc*-tag (Munro and Pelham, 1986) for detection, as well as a C-terminal histidine tail for purification (Ge *et al.*, 1995; Lindner *et al.*, 1992). Thus, the amount of soluble periplasmic protein could be determined by Western blotting with two different antibodies, and it was

A

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
													FR1																				CDR1											FR2	
93–6	E	V	K	L	V	E	S	G	A	E	L	V	R	P	G	T	S	V	K	M	S	C	K	A	A	G	Y	T	F	T	N	Y	Y	I	G	W	V	K	Q	R	P	A	H	G	L

	46	47	48	49	50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	82A	82B	82C	83	84	85	86
												CDR2																								FR3									
93–6	E	W	I	G	D	I	Y	P	G	G	G	Y	P	N	Y	N	E	K	F	K	G	K	A	T	L	T	A	D	T	S	S	S	T	A	Y	M	Q	L	S	S	L	T	S	E	D

	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113
												CDR3										FR4					
93–6	S	A	I	Y	Y	C	A	R	S	G	V	F	D	Y	–	–	W	G	Q	G	T	T	L	T	V	S	S

B

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	27A	27B	27C	27D	27E	27F	28	29	30	31	32	33	34	35	36	37	38	39
								FR1																								CDR1													
muKL I	D	I	V	M	T	Q	S	P	S	S	L	A	V	S	A	G	E	K	V	T	M	S	C	K	S	S	Q	S	L	L	N	S	G	N	Q	K	N	Y	L	A	W	Y	Q	Q	K
muKL II	D	V	V	M	T	Q	T	P	L	S	L	P	V	S	L	G	D	Q	A	S	I	S	C	R	S	S	Q	S	L	V	H	S	–	N	G	N	T	Y	L	E	W	Y	L	Q	K
muKL III	D	I	V	L	T	Q	S	P	A	S	L	A	V	S	L	G	Q	R	A	T	I	S	C	R	A	S	E	S	V	D	S	–	–	Y	G	N	S	F	M	H	W	Y	Q	Q	K
muKL IV	E	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	G	E	K	V	T	M	T	C	S	A	S	S	S	–	–	–	–	–	V	S	S	S	Y	L	H	W	Y	Q	Q	K
muKL V	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	L	G	D	R	V	T	I	T	C	R	A	S	Q	–	–	–	–	–	–	D	I	S	N	Y	L	N	W	Y	Q	Q	K
muKL VI	Q	I	V	L	T	Q	S	P	A	I	M	S	A	S	P	G	E	K	V	T	M	T	C	S	A	S	S	–	–	–	–	–	–	–	S	V	S	Y	M	H	W	Y	Q	Q	K
93–6	D	I	E	I	T	Q	S	P	A	S	L	A	V	S	L	G	Q	K	A	T	I	S	C	K	A	S	K	E	V	T	I	F	–	G	S	I	I	A	L	H	W	Y	Q	Q	K

	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	69A	69B	69C	69D	69E	69F	69G	69H	70	71	72	73	74	75	76
			FR2											CDR2																			FR3												
muKL I	P	G	Q	S	P	K	L	L	I	Y	W	A	S	T	R	E	S	G	V	P	D	R	F	T	G	S	G	S	G	T	–	–	–	–	–	–	–	–	D	F	T	L	T	I	S
muKL II	P	G	Q	S	P	K	L	L	I	Y	K	V	S	N	R	F	S	G	V	P	D	R	F	S	G	S	G	S	G	T	–	–	–	–	–	–	–	–	D	F	T	L	K	I	S
muKL III	P	G	Q	P	P	K	L	L	I	Y	A	A	S	N	L	E	S	G	V	P	A	R	F	S	G	S	G	S	G	T	–	–	–	–	–	–	–	–	D	F	T	L	N	I	H
muKL IV	P	G	X	S	P	K	L	W	I	Y	R	T	S	N	L	A	S	G	V	P	A	R	F	S	G	S	G	S	G	T	–	–	–	–	–	–	–	–	S	Y	S	L	T	I	S
muKL V	P	G	G	S	P	K	L	L	I	Y	Y	A	S	R	L	H	S	G	V	P	S	R	F	S	G	S	G	S	G	T	–	–	–	–	–	–	–	–	D	Y	S	L	T	I	S
muKL VI	S	G	T	S	P	K	R	W	I	Y	D	T	S	K	L	A	S	G	V	P	A	R	F	S	G	S	G	S	G	T	–	–	–	–	–	–	–	–	S	Y	S	L	T	I	S
93–6	P	G	Q	P	P	K	L	–	I	Y	N	G	A	K	L	E	S	G	V	S	A	R	F	S	D	S	G	S	Q	N	R	S	P	F	G	N	Q	L	N	F	T	L	T	I	D

	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
																	CDR3											FR4					
muKL I	S	V	Q	A	E	D	L	A	V	Y	Y	C	Q	N	D	Y	S	Y	P	L	T	F	G	A	G	T	K	L	E	L	K	R	A
muKL II	R	V	E	A	E	D	L	G	V	Y	Y	C	F	Q	G	T	H	V	P	Y	T	F	G	G	G	T	K	L	E	I	K	R	A
muKL III	P	V	E	E	D	D	A	A	T	Y	Y	C	Q	Q	S	N	E	D	P	W	T	F	G	G	G	T	K	L	E	I	K	R	A
muKL IV	S	M	E	A	E	D	A	A	T	Y	Y	C	Q	Q	W	S	S	Y	P	L	T	F	G	A	G	T	K	L	E	I	K	R	A
muKL V	N	L	E	Q	E	D	I	A	T	Y	F	C	Q	Q	G	N	T	L	P	R	T	F	G	G	G	T	K	L	E	I	K	R	A
muKL VI	S	M	E	A	E	D	A	A	T	Y	Y	C	Q	Q	W	S	S	N	P	L	T	F	G	A	G	T	K	L	E	L	K	R	A
93–6	P	V	E	A	D	D	A	A	T	Y	Y	C	L	Q	N	K	E	V	P	Y	T	F	G	G	G	T	K	L	E	I	K	R	A

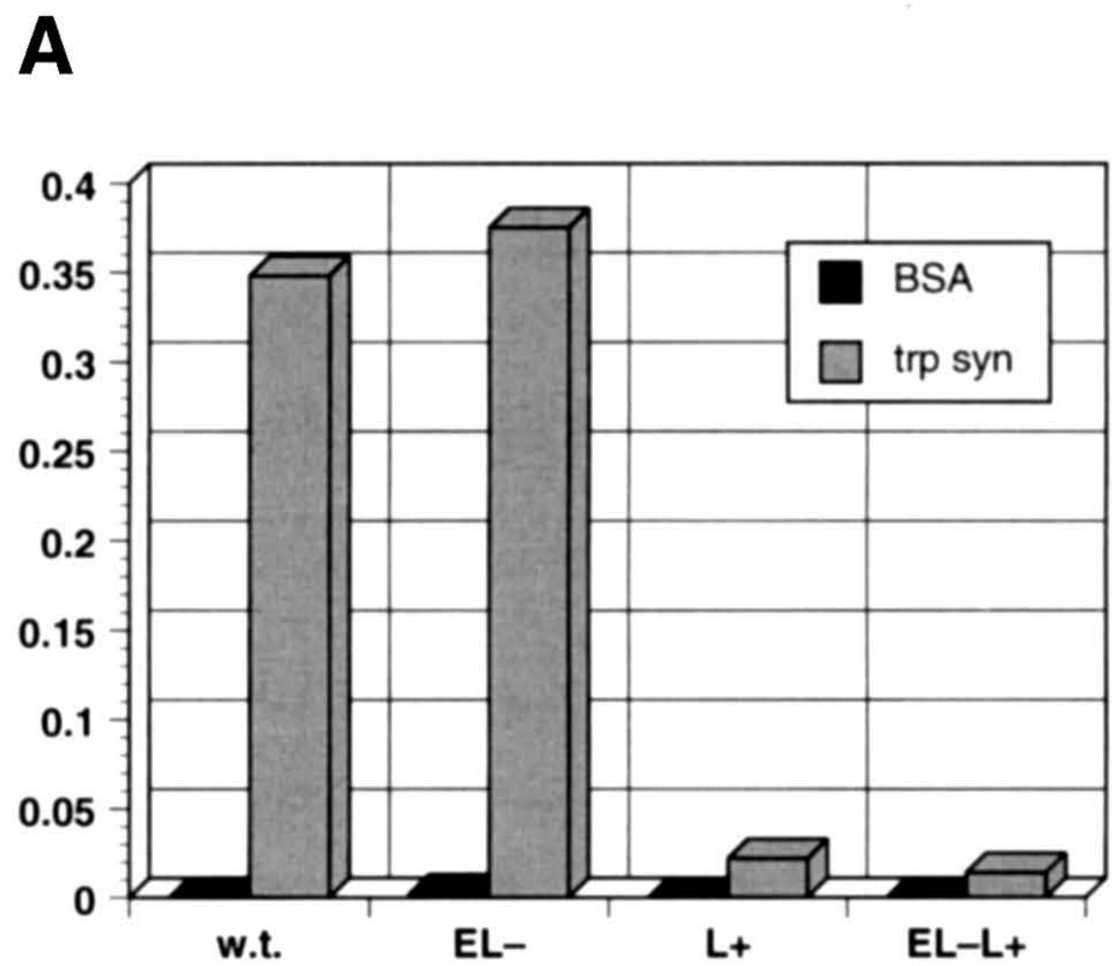
FIG. 1. *A*, Sequence of the heavy chain of the antibody 93-6 and *B*, sequence of the light chain, aligned to the consensus sequences of the six classes of mouse κ chains as defined by Kabat *et al.* (1991). Note that in both chains the first two amino acids are encoded by the PCR primer and the true residues are therefore unknown. The numbering according to Kabat is indicated. *X* indicates a variable residue with no clear preference, the dash (–) a deletion. The insertion and the deletion in the framework of 93-6 are highlighted.

found to be very similar for all four variants (data not shown).

In an antigen binding assay of *E. coli* periplasmic extracts, only the wild type protein and the EL$^-$ mutant gave a positive signal on an ELISA plate coated with tryptophan synthase β-subunit (Fig. 2*A*). Similar results were obtained with purified proteins. The binding constant for the wild type and the EL$^-$ version was then determined in solution (Friguet *et al.*, 1993), using luminescence ELISA as detection method. The binding constant determined for the wild type and EL$^-$ were different only by a factor 3 (Fig. 2*B*), suggesting that the extra loop does not make significant contact to the antigen. The failure of the two leucine insertions L$^+$ and EL$^-$L$^+$ to bind antigen, observed with both purified protein and crude periplasmic extracts, was more unexpected, and may indicate a crucial structural change in CDR2, normally extremely well conserved. While all the mutant scFv fragments are soluble in the periplasm, some aggregation of the purified protein was observed upon concentration, but this appears to correlate more with the presence of the extra loop than the Leu insertion.

To obtain a better insight into the potential effects of these mutations on the structure of 93-6, we built a molecular model for the Fv fragment (Fig. 3) using standard homology modeling techniques (see "Materials and Methods"). As anticipated from the binding studies, the insertion appears to be too far removed from the binding pocket to interact efficiently with the antigen. Its involvement in binding is further hindered by the screening effect of the large CDR L1. Nevertheless, in future projects, a combination of a FR3 insertion with a short CDR L1 may become interesting for engineering antibodies that bind large flat epitopes. A 4-amino acid insertion into the FR3 loop of the heavy chain of an anti-nitrophenyl antibody has also been constructed and found to not influence activity (Simon and Rajewsky, 1992), and this would not be expected for a hapten-binding antibody, but such an insertion could again be useful for a large flat antigen.

The deletion at residue 47 lies in a structurally conserved β-bulge that is also found in antibody heavy chains, and in CD2, CD4, and CD8 (Chothia *et al.*, 1985; Jones *et al.*, 1992; Wang *et al.*, 1990; Leahy *et al.*, 1992), despite great sequence divergence in this area and different lengths of the following loop. The deletion could potentially be accommodated in two ways: (i) the β-bulge could be maintained by moving Tyr-49 into the protein interior and relaxing the unusual γ-turn found in CDR L2 structures into a more common type II′ β-turn or (ii) the β-bulge could be resolved into a normal β-strand, leaving the γ-turn in CDR L2 intact but causing a shift in the following framework loop to compensate for the missing residue in the core. Both possibilities yielded plausible models (Fig. 3*D*), with the second obtaining slightly better scores in the three-dimensional-one-dimensional validation method of Lüthy *et al.* (1992). Neither structural model provides a clear indication why the insertion of the missing leucine has such a dramatic



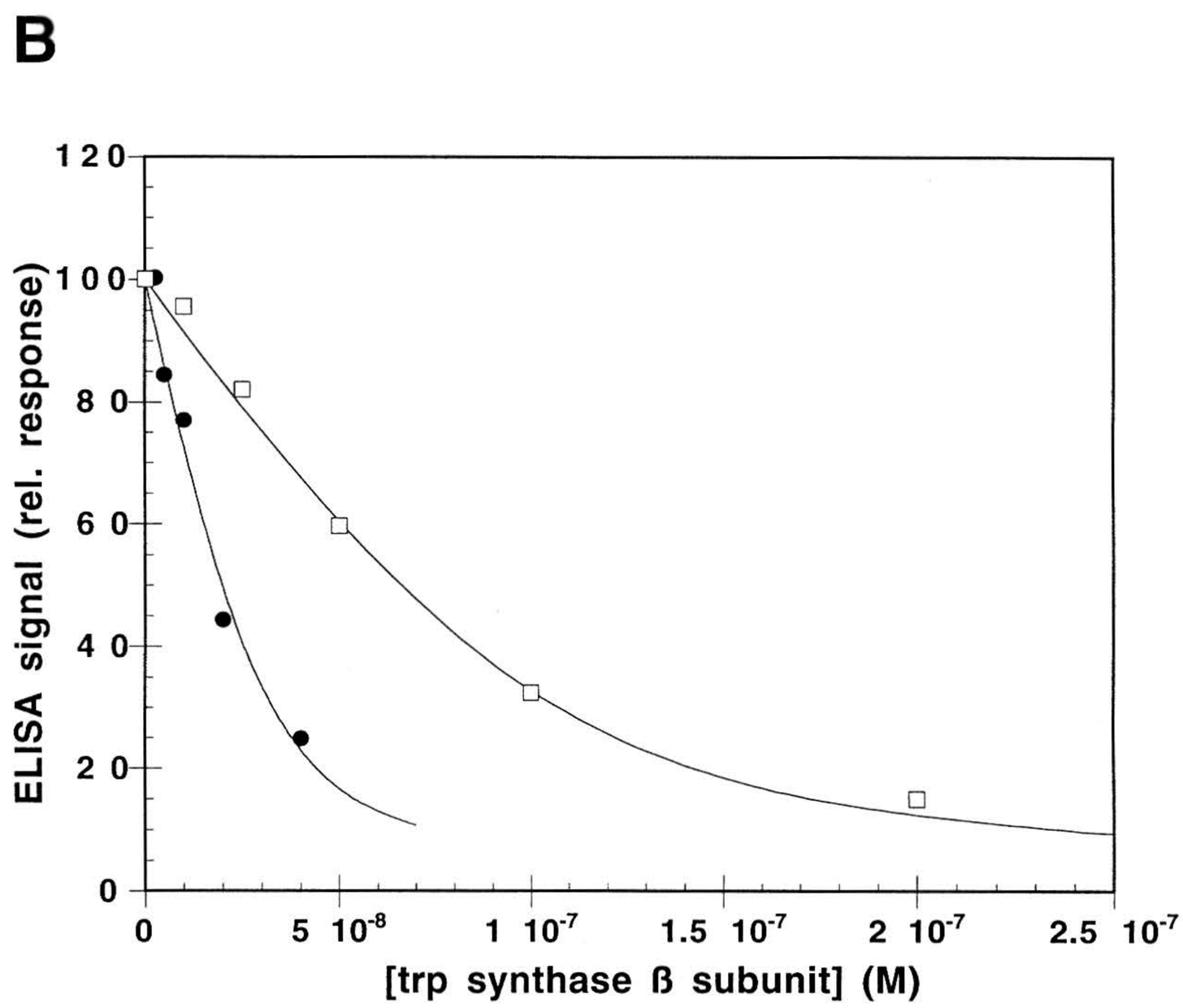


FIG. 2. *A*, periplasmic extracts of the mutants of 93-6 scFv fragments were analyzed by luminescence ELISA with the relevant antigen tryptophan synthase, and bovine serum albumin was used as a negative control. The concentration of soluble scFv fragments was estimated from Western blots (Ge *et al.*, 1994; Knappik and Plückthun, 1994) and the amounts of periplasmic extracts used in the ELISA were adjusted to correspond to identical scFv concentrations. The height of the bars is given in arbitrary light units. *B*, determination of the binding constant of the scFv fragments of the antibody 93-6 (wild type) (●) and the EL^- mutant (□) by the method of Friguet *et al.* (1985). From two protein preparations, K_d of the wild type was found to be $5.3 \cdot 10^{-9}$ M and $1.6 \cdot 10^{-8}$ M for the EL^- mutant. The K_d of the non-recombinant F_{ab} is $6.7 \cdot 10^{-9}$ M (Brelier and Goldberg, 1990).

effect, but it must be considered that the deletion in 93-6 may have caused far-reaching repacking of the hydrophobic core, ultimately influencing the conformation of several CDRs, while homology modeling by its very premise attempts to compensate for all changes locally.

A more extensive repacking is supported by the fact that two other highly conserved residues of mouse κ chains are mutated in 93-6, and both are located in the same part of the V_L structure as the framework deletion, namely in the region outlined by strands C′, C″, and D: Pro-59, which in other κ chains determines the structure of the loop connecting strands C″ and D, is replaced by serine in 93-6, and Gly-64, which in other κ chains faces an internal conserved water molecule, is replaced by aspartate. The latter residue in particular, which in our model replaces the internal water with its side chain and assumes its interactions with the central tryptophan (Trp-35), may instead cause a distortion in strand D that would reorient the charged side chain to the outside and allow its solvation.

An attempt was made to find the correct docking orientation of 93-6 to tryptophan synthase by analyzing the electrostatic potential of the two molecules in the DELPHI module of the INSIGHT II modeling package. This approach was not successful as both molecules showed a patchy distribution of positive and negative potentials without clear complementarity. Nevertheless, an approximate docking orientation can be derived from steric considerations: almost all orientations other than an almost perpendicular orientation of the V_H-V_L axis relative to the $\alpha\beta\beta\alpha$ axis are unlikely because of significant overlaps. An interaction of the insertion with tryptophan synthase only appears likely if V_L is oriented toward the flat side of the β_2 dimer.

It is currently unclear by which mechanism the two deviations from the consensus κ structure came about. Insertions and deletions are extremely rare in the somatic maturation of antibodies (Chen and Poljak, 1974; Press and Hogg, 1970; Wang *et al.*, 1973; Cumano and Rajewski, 1986), and in the the present antibody, two such events would be required. Since, in contrast to the human sequences, only a fraction of the mouse κ genes are known, it remains a possibility to be investigated in future work whether an unusual Vκ gene has remained undiscovered.

In conclusion, this functional antibody shows that conserva-

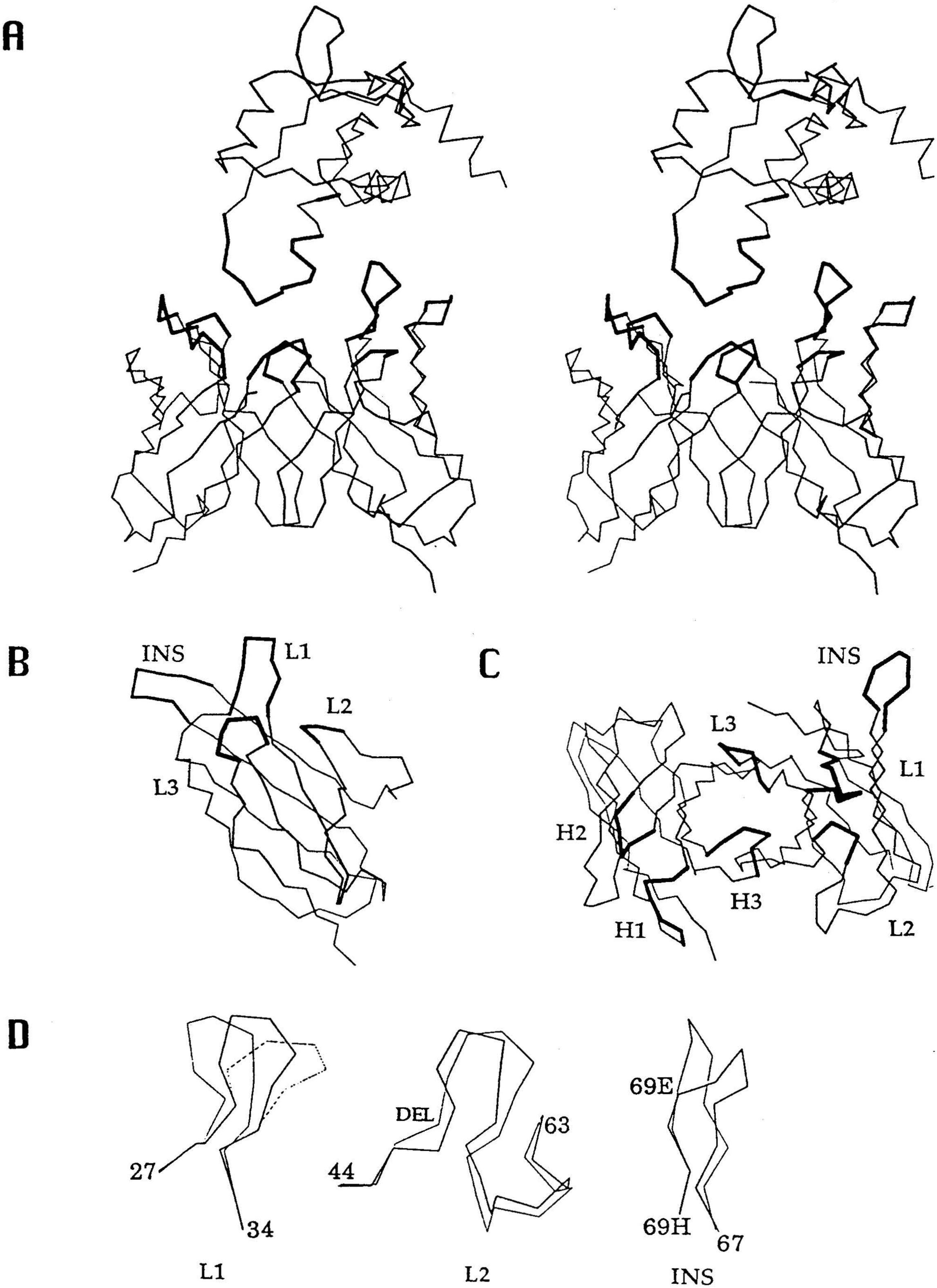


FIG. 3. *A*, stereo view of a model for the interaction of antibody 93-6 with fragment F2 of the tryptophan synthase β-subunit (1WSY residues B276–B393). The six CDR loops, the light chain insertion and the solvent-exposed residues of the F2 epitope mapped by Goldberg and co-workers (Friguet *et al.*, 1989, 1993) are shown in *bold*. *B*, view of the light chain across the binding pocket and *C*, top view of the 93-6 binding pocket. CDR loops (*L1*, *L2*, *L3*, *H1*, *H2*, and *H3*) and insertion (*INS*) are shown in *bold*. *D* (*left*), CDR L1 as modeled on 1IMN is compared to CDR L1 of 1IGF

tion of consensus structure is not necessary for function. On the contrary, conversion to the consensus in FR2 makes the antibody unable to bind its antigen. We suggest therefore that even those CDRs which are restricted in length, as well as adjacent stretches of framework residues, should be included in antibody mutagenesis schemes, as the consensus framework may provide only a subset of potentially good binders.

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(*thin line*) and residues 17–23 of 1SNC (staphylococcal nuclease; *dotted line*); (*middle*) CDR L2 with the preceding β-bulge preserved and the γ-turn relaxed into a β-turn is compared to CDR L2 with the β-bulge resolved into a continuous β-strand and the γ-turn preserved (*thin line*); (*right*) the most reclined and the most upright insertion conformation identified from PDB by the method of Jones and Thirup (1986). The variability stems from the conformational freedom of Gly-69E (see Fig. 1) at the base of the turn.