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Predicting local structural changes that result from point mutations

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Point mutations are frequently used to explore the structure and/or function of proteins. The ability to predict the structural effects of point mutations would make the planning of such experiments more reliable. We have now derived a set of detailed predictive rules based on the comparison of crystal structures of point mutants and wild types in 83 cases. Despite the surprising simplicity of these rules, they describe well the conformational changes in 85% of all point mutant structures available at present.
Key words: point mutations/predictive rules/protein structure

Introduction

A single mutation in a protein can dramatically influence its stability (Dao-pin *et al.*, 1991a,b,c,d) or function (Grenzin *et al.*, 1992). However, large structural changes as a result of one single mutation are rare (Thunissen *et al.*, 1991). Generally, the overall structure of proteins does not change upon the introduction of a point mutation. If a mutated amino acid does not fit well into its environment, conformational adaptations are made mainly by the mutated residue, to a lesser extent by its entire secondary structure element (Laughton, 1994) and rarely by its 3-D contact partners.

Prediction of the structure of a mutated protein is a prerequisite for the design of point mutations aimed at altering the functional characteristics of a protein. Unfortunately, calculations or even estimates of free energy changes are insufficiently accurate to be used as a routine tool for predicting structural changes. On the other hand, empirical methods based on a variety of approaches work quite well for some purposes (Lee and Levitt, 1991; Simonson and Brunger, 1992; van Gunsteren and Mark, 1992; Totrov and Abagyan, 1994). However, a reliable and general theory of protein conformational changes does not exist to date.

To circumvent the difficulties with energy calculations, we exploit the fact that certain local structural features are repeated many times in different protein structures. Intuitively, the frequent occurrence of features in otherwise different proteins implies that these characteristics are beneficial for folding and stability, and/or that they are easily reached in an evolutionary mutation–selection process. By extrapolation, such features can be used for the prediction of conformational changes as a result of point mutations.

We have therefore developed a statistical method for predicting structural changes that is based on searches in the database of 3-D structure. Given a residue to be mutated, we search the database for residues with a similar environment and then assess which rotamers in the database are statistically preferred and sterically admissible in the current structure. Using simple rules based on this scheme, the local structural environment of a point mutation can be predicted correctly in the majority of all cases. The method is based on simple statistical arguments and does not require time consuming energy calculations.

Materials and methods

A variety of methods in the program WHAT IF (Vriend, 1990) were used to analyse 83 high-resolution X-ray mutant structures: 54 T4 lysozymes, five human lysozymes, eight carbonic anhydrases, two interleukin-1βs, two isocitrate dehydrogenases, three chloramphenicol acetyl transferases, two α-lytic serine proteases, one ferredoxin, one staphylococcal nuclease, one renin, one cytochrome P-450, one Ras-p21 GTPase domain and two arabinose binding proteins. Tables I and II summarize the analysed structures. We only analysed mutant structures that were crystallized under similar conditions to the wild type. X → Gly, Gly → X, X → Pro and Pro → X mutations were excluded when considerable backbone changes were observed. Several mutants [e.g. E46Q in RNase T1 (Grenzin *et al.*, 1992) and C20A in ferredoxin (Stout, 1989)] were not used because of an altered mode of binding of a cofactor. Several other mutants were not included because the wild type and mutant structures were determined by nuclear magnetic resonance, and showed too many small differences (e.g. K47E in hirudin; Folker *et al.*, 1989).

For every mutant a position-specific rotamer analysis was performed. This technique has been described in detail elsewhere (Jones and Thirup, 1986; Vriend and Eijsink, 1993; Vriend *et al.*, 1994). Briefly, a rotamer distribution at a certain position is determined by extracting from a database of non-redundant protein structures (Hobohm *et al.*, 1992) all suitable fragments of five or seven residues in length (seven in helix and strand, five in the case of irregular local backbone). Suitable fragments are those that have a local backbone conformation similar to that around the evaluated position, and have the same residue type at the central position. In these analyses, the r.m.s. deviation of the C_α positions between the structure and the database fragment was kept <0.5 Å. The rotamer distributions were then used to answer the following questions: (i) what are the differences between the rotamer of the residue mutated by protein engineering and the position-specific rotamer distribution obtained from the database of natural proteins? and (ii) which set of rules would have allowed us to predict the structure of the mutated residue correctly?

Results and discussion

Several rotamer libraries have been described (McGregor *et al.*, 1987; Ponder and Richards, 1987; Morris *et al.*, 1992; Dunbrack and Karplus, 1994). In most studies the χ_1 distribution of the 20 residues was analysed as a function of secondary structure. In some cases the ends of secondary structure elements were treated separately. The position-

Table I. General mutants used in this study

Mutation	sg	el	ei	nRP	RPD χ_1	RDH χ_2, χ_3, χ_4	dssp	access	Predet
1L17,Ile3Val	0.1783	0.33	0.28	2	2:1		H	3 67	yes[c]
1L18,Ile3Tyr	0.3355	0.84	0.65	3	4 2 1	5 1	H	"	yes[b]
1L19,Ser38Asp	0.2035	0.40	0.14	3	4:2.1	3:1		11.53	yes[a]
1L42,Lys16Glu	0.3053	**1.54**	0.15	–	–		E	15 03	noC
1L43,Lys16Glu	0.2781	**1.54**	0.08	–	–		E	"	[d]
1L34,Arg96His	0.2084	0.35	0.21	2	3·1	4:1	H	3 49	yes[a]
1L48,Ala98Val	0 3231	0.60	0.46	1	–		H	0 00	yes
1L54,Met102Lys	0 2663	**2.87**	0.15	2	3 1	3:1, 3 1, 2·1	H	2 97	yes
1L57,Asn116Asp	0.4492	0.25	0.28	3	3.2 1	2 1	H	9 26	yes[a]
1L44,Arg119Glu	0.2767	0.25	0.13	2	2 1	2:1, 5.1	H	11.01	yes
1L45,Lys135Glu	0.1298	0 24	0.10	–		D	S	14.68	no
1L20,Asn144Asp	0.2581	0 09	0.17	3	2 1:1	2:1	H	16 42	yes
1L46,Lys147Glu	0.2720	0 19	0 13	2	3 1	3 1, 4 1	H	17.71	yes[a]
1L53,Val149Cys	0.4980	0 60	0.31	2	3 1		H	0 17	yes[c]
1L52,Thr152Ser	0 2311	0.29	0 10	3	3:1.1		H	0.52	yes
1L47,Arg154Glu	0.3055	**4.18**	0 12	2	2 1	2 1, 3 1	H	5 77	yes
1L16,Gly156Asp	0.5067	0 80	0 63	–	D		S	4.54	[d]
1L04,Thr157Asp	0.4789	0.66	0 34	3	3 2 1	2 1	S	9 44	yes
1L05,Thr157Asp	0.4621	0 66	0 28	3	4·2 1	4:1 "	S	"	yes[a]
1L06,Thr157Glu	0.4811	0 28	0 14	–	D		S	"	[d]
1L07,Thr157Phe	0.4821	0 47	0 16	3	3:2 1	4·1	S	"	yes
1L09,Thr157His	0.4772	0.50	0.24	3	3:2 1	5 1 "	S	"	yes[c]
1L10,Thr157Ile	0.4800	**1.43**	0.25	2	2:1	4:1	S	"	yes
1L11,Thr157Leu	0.4564	0.76	0.21	2	5 1	5:1	S	"	yes
1L12,Thr157Asn	0 4601	0.29	0.31	3	3 2·1	2:1	S	"	yes
1L13,Thr157Arg	0.4972	0.23	0.10	1	1	1, 1, 1	S	"	yes
1L14,Thr157Ser	0.4743	0.34	0.07	3	4 2 1		S	"	yes
1L15,Thr157Val	0.4791	0.80	0.20	2	2 1		S	"	yes
1L27,Pro86Asp	0 1286	0.90	0.19	2	5:1	4 1	H	12 23	yes
1L29,Pro86His	0.2361	**1.41**	0.24	2	3 2	2:1	H	"	yes
1L30,Pro86Leu	0 2384	0.90	0 24	2	4 1	3·1	H	"	yes
1L31,Pro86Arg	0 4824	**1.44**	0.38	–	D		H	"	noC
1L32,Pro86Ser	0.2516	**1.54**	0.24	–	D		H	"	noC
1L55,Asp92Asn	0.4724	0.54	0.26	2	4 1	3 1		13.11	yes
1L61,Ser38Asn	0.3882	0 48	0.16	3	5:2:1	3 1		11 53	yes[a]
1L62,Thr109Asp	0.4086	0 66	0 12	3	4 2:1	3:1	H	20.27	yes
1L59,Thr109Asn	0.4110	0.56	0.06	3	2:1.1	2 1	H	"	yes
1L37,Thr115Glu	0.2233	0 66	0 22	–	D		H	16.25	[d]
1L38,Gln123Glu	0.2985	**2.51**	0 05	2	3 1	4.1, 3 1	T	8.74	yes[a]
1L39,Asn144Glu	0.2586	0 53	0.24	2	3·1	3 2 1, 3 1 "	H	16.43	yes[a]
1L40,Asn144Glu	0.2561	0 48	0 24	2	2 1	4:2:1, 3 1	H	"	yes
1LAA,Asp53Glu	0.5071	**4.26**	0 16	3	4 2 1	3 1, 2 1	E	3 14, 9 96	yes[a]
1TBY,Tyr63Leu	0.5116	**1.46**	0 17	1		2:1	T	"	yes
1TCY,Tyr63Phe	0.4892	0 74	0.06	3	8 2 1	5 1	T	"	yes
1TDY,Tyr63Trp	0.4798	0.76	0.14	3	4:2:1	3 1	T	"	yes
5CA2,Thr200Ser	0.3491	**2.62**	0.40	3	3.2 1		S	6 29	yes
6CA2,Val143Phe	0.3736	**1.23**	0 27	3	4·2 1	4·1	E	2 1	yes[b]
8CA2,Val143His	0.3458	0.73	0.29	3	5:2:1	2 1	E	2.1	yes
9CA2,Val143Tyr	0.3603	**1.07**	0 28	3	3 1:1	4:1	E	"	yes TR
1HEA,Leu198Arg	0.3583	0.60	0.18	–	D			8.97	noC
1HEC,Leu198His	0.2777	0 86	0.31	–	D			"	noC
6ICD,Ser113Asp	0.2461	0 80	0 09	3	8 1 1	5 1		11.7	yes[a]
7ICD,Ser113Glu	0.2421	0 52	0.28	–	D			"	noC
4CLA,Leu160Phe	0.2140	**1.05**	0.09	3	4.2 1	5 1		7 5	yes
2CLA,Asp199Asn	0.4006	**5.62**	0.66	2	4 1	5 1		9 3	yes
2Q21,Gly12Val	0.4915	**1.22**	0 13	2	3 1		T	19 4	yes[c]
3CMS,Val111Phe	0.7926	**2.45**	0.18	3	4:2 1	4.1	H	4 4	yes
2SNM,Val66Lys	0.5846	**2.44**	0.14	2	3:2	2:1 "	H	2.8	yes TR
6ABP,Met108Leu	0.4185	0 60	0.40	2	3.1	2:1	E	5 2	yes TR

References for mutants used in this study are Grutter *et al.* (1979, 1987), Gilliland and Quiocho (1981), Wlodawer *et al* (1984), Alber *et al* (1987, 1988), Bone *et al* (1987), Gray and Matthews (1987), Matsumura *et al* (1988, 1989), Nicholson *et al* (1988, 1989, 1991), Finzel *et al* (1989), Kitamura and Sturtevant (1989), Matsumura and Matthews (1989), Weaver *et al* (1989), Dao-pin *et al.* (1990, 1991a,b,c,d), Hurley *et al* (1990), Lewendon *et al* (1990), Loll and Lattman (1990), Strop *et al* (1990), Alexander *et al.* (1991), Krebs *et al.* (1991), Murray *et al* (1991), Nair *et al* (1991), Raag *et al* (1991), Stites *et al* (1991), Zhang *et al* (1991), Harata *et al.* (1992), Heinz *et al.* (1992) and Muraki *et al* (1992).

[a]The higher χ was modified to improve hydrogen bonding

[b]χ_1 differs up to 20° from the preferred position-specific rotamer

[c]Side chain in second best position-specific rotamer because of bump in best rotamer

[d]Poor statistics. Structure prediction often correct, but poorly reproducible. In the case of long charged side chains, the polar atoms are normally placed more accurately than the hydrophobic ones.

noC, poor statistics and mispredicted, no, misprediction, D, very diffuse rotamer distribution; yes, predicted correctly using the most populated rotamer, TR, there is only one way to accommodate the new side chain without bumps, thus trivial prediction; sg, r.m.s. deviation of all atoms after superposing wild type and mutated protein, el, largest atomic movement in the environment of the mutated residue (changes >1 Å are in bold); ei, largest atomic movement in the backbone of the mutated residue; nRP, number of observed distinct populations in χ_1; RPD, relative frequency of occurrence in the nRP populations in χ_1, RDH, relative frequency of occurrence in distinct populations of χ_2, χ_3, χ_4 (only given if applicable). In RPD and RDH a 1 indicates that only one distribution was observed A D indicates a very diffuse distribution. dssp, secondary structure classification according to DSSP (Kabsch and Sander, 1983), access, accessible surface area of β position for the amino acid side chain after mutating it into an alanine.

Table II. X → Ala and X → Gly mutants

Mutation	sg	el	ei	dssp	access
1L33,Val131Ala	0.2272	0.66	0.19	H	14.15
1L69,Leu133Ala	0 4243	0.85	0.27	H	3.14
1L02,Thr157Ala	0.4647	0.33	0.15	S	9.44
1L58,Pro143Ala	0 2379	0.42	0.44	H	8.34
1L21,Asn55Gly	0.2318	0 30	0.38	T	16.95
1L08,Thr157Gly	0.4604	0.39	0.23	S	9.44
1L22,Lys124Gly	0.2180	0.40	0.23	T	11.53
1L66,Lys43Ala	0.4466	0 42	0.16	H	8.91
1L67,Leu46Ala	0.1750	0 39	0.20	H	0.52
1L68,Ser44Ala	0.4475	0.56	0.25	H	14.50
1L65,Asp47Ala	0.4833	**1.56**	0.08	H	7.70
1L25,Pro86Ala	0.2686	**1.62**	0.21	H	12.23
1L28,Pro86Gly	0.1747	**1.35**	0.22	H	
1P08,Met192Ala	0.1459	**1.70**	0.08		1.20
1P09,Met213Ala	0.3160	**4.49**	0.14	E	0.00
21BI,Cys71Ala	0.6015	0.86	0.20	E	0.20
41BI,Cys8Ala	[e]	**3.37**	0.08	E	0.00
2FD2,Cys24Ala	0.2313	0 60	0.45		0 20
1CLA,Ser148Ala	0.1547	**1.37**	0.09	E	8.00
2CP4,Thr252Ala	0.3763	0 90	0.70	T	8 40
1TAY,Tyr63Ala	0 4863	**1.58**	0 23	T	9.90
12CA,Val121Ala	0.2671	0 63	0.09	E	3 50
7CA2,Val143Gly	0.3632	0 52	0.48	E	2 10
1APB,Pro254Gly	0.4113	0 54	0.57	E	0.50

[e], too many side-chain atoms missing in mutant structure to determine r.m.s. deviation

See footnote to Table I for key to table and references.

specific rotamer distributions used here allow for different rotamer distributions at each position and thus provide a more refined description than do standard rotamer libraries. This is especially true near the ends of secondary structure elements (Vriend *et al.*, 1994).

X → Ala and X → Gly mutations

We have analysed the structural consequences of 17 X → Ala and six X → Gly mutants, where X is a more voluminous residue. In these cases one can be sure that no strain is introduced by the mutation. If major changes in the structure around the mutated residue were observed then this would be a strong indicator of strain in the environment of the mutated residues. All 24 mutants of this kind found in the June 1993 version of the Protein Data Bank (PDB; Bernstein *et al.*, 1972) are listed in Table II. One rarely sees large rearrangements of the residues in the direct environment. Seldom does χ_1 change by >10–20° (except in two cases, PDB files 1L47 and 1LAA, where a glutamic acid rotates by ~160° to make a hydrogen bond). The only three residues that showed significant rearrangements in these 23 cases are methionines. It is tempting to try to derive residue-specific rules, but the number of examples is still far too small for such an analysis to be statistically significant. Recently Buckle *et al.* (1993) made five mutations in the hydrophobic core of barnase. In all five cases a residue was replaced by a similar but smaller one, and they observed that the mutated residue moves to the greatest extent normally in the direction of the cavity.

In the cases of the X → Ala and X → Gly mutations, little change is observed in the direct environment of the mutated residue. This trend extends to other types of mutation. Almost all big changes introduced by point mutations arise from residues with long side chains (arginine, glutamic acid, etc.) at the protein surface (Tables I and II). The side chains of these residues can move around freely and are often involved in salt bridge breakage or formation.

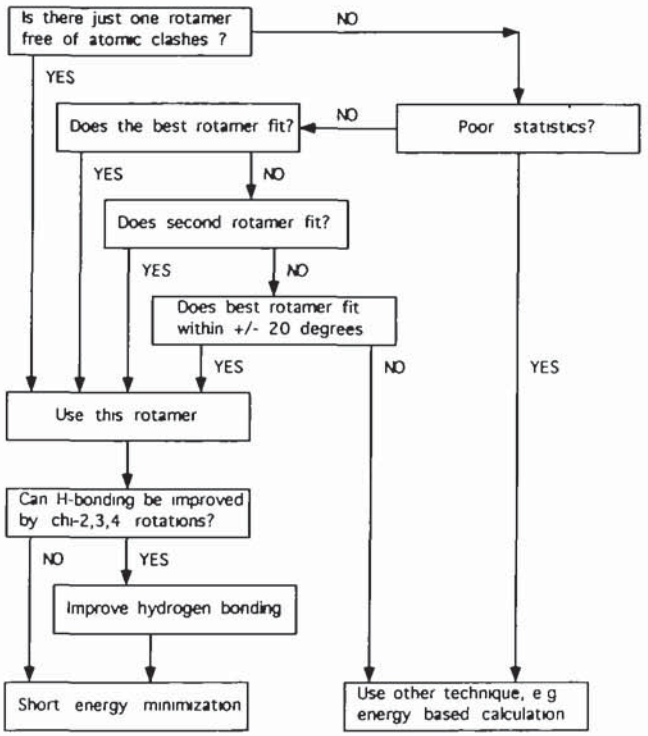

Fig. 1. Decision schema for the prediction of point mutant structures.

Other mutations

In the analysis of all mutations we observed six classes of mutational behaviour. (i) There is only one way of placing the residue without atomic clashes. In all four examples in this class we find only one rotamer that leaves the rest of the molecule unaltered and this is the observed rotamer. (ii) The conformation of the mutated residue corresponds to the most populated position-specific rotamer. This occurs in ~50% of all cases. (iii) The most populated rotamer would lead to atomic clashes and the conformation of the mutated residue corresponds to the second most populated rotamer (four examples). (iv) Neither the most populated nor the second most populated rotamer fits, and the conformation of the mutated residue is very close to the most populated rotamer (two examples found, both deviate ~20° from the optimal χ_1). We never observed that the third, i.e. least populated, rotamer was selected. (v) The χ_1 of the mutated residue corresponds to the most populated rotamer, but the χ_2 or χ_3 angle is rotated away from the optimal position-specific rotamer distribution to optimize hydrogen bonding. This is observed in almost 15% of all cases (10 examples). (vi) There is no preferred position-specific rotamer, or there are insufficient examples in the database, but analysis of potential hydrogen bonds leads to one clear possibility for the structure. This occurs in 7% of all cases (four examples). Although in these cases it is possible to fix the charged end of the side chain in the proper position, it is often difficult to predict accurately the correct conformation of the hydrophobic part of the side chain.

We have converted these six observed classes into a set of rules. In Figure 1 we show a scheme for the prediction of the structure of mutated residues based on these rules. If we assume that the present data set (Tables I and II) is representative of the universe of mutated residues, then this scheme allows us to predict correctly the local structures of at least 85% of all mutated residues. The actual predictive ability on proteins not studied here may, of course, be lower.

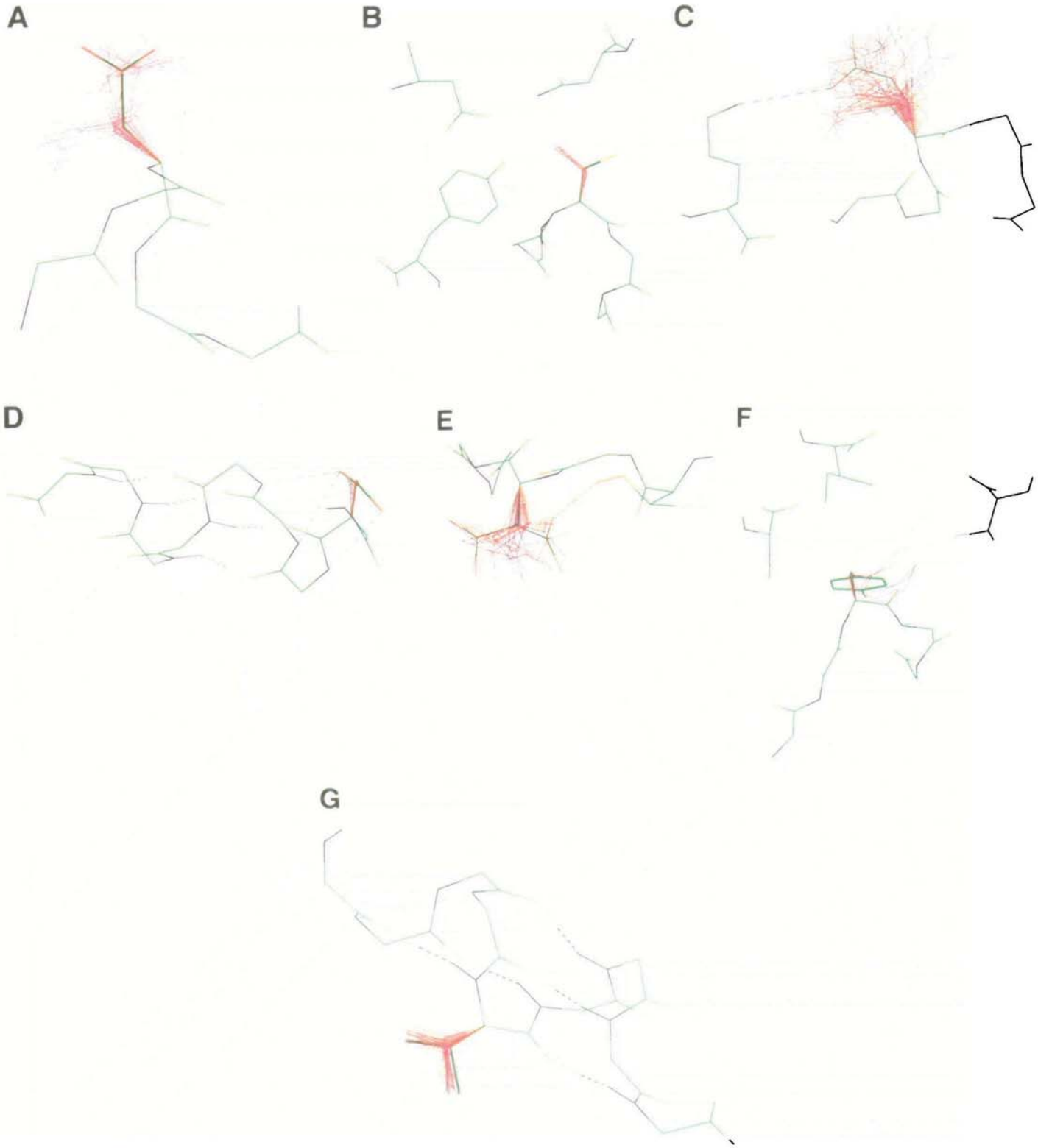

Fig. 2. Some examples of database-derived rotamer distributions superposed on the experimentally determined structure. The experimental rotamer and the backbone of the five residue fragment on which it is centred are shown (red = oxygen, blue = nitrogen, yellow = sulfur, green = carbon). The database-extracted rotamers are coloured blue to red for best fitting to poorest fitting, respectively. Where applicable, residues in the direct environment are also displayed. (**A**) Two rotamers observed, most populated rotamer selected. Thr109 → Asp in lysozyme (PDB file 1L62). This example is representative of the largest number of cases. (**B**) Best rotamer shows atomic clashes, second best rotamer selected. Val149 → Cys in lysozyme (PDB file 1L53). (**C**) Rotamers too diffuse, salt bridge (green dashed line) determines position. Thr115 → Glu in lysozyme (PDB file 1L37). (**D**) χ_1 correct, χ_2 rotates away to optimize hydrogen bonding (indicated by green dashed lines). Ser38 → Asp in lysozyme (PDB file 1L19). The backbone of the helix capped by this residue is shown, and intra-helix hydrogen bonds are indicated by dashed lines. Five out of eight residues have the same χ_1, but four of those have a χ_2 angle 30–60° away from the experimentally observed rotamer. (**E**) Special case. Best rotamer for Thr157 → Asp is observed in PDB file 1L04. A rotation of χ_2 would lead to an extra hydrogen bond with a Thr two residues away in the sequence. This is indeed observed as an alternative conformation in PDB file 1L05. The Thr and the hydrogen bond are shown too. (**F**) χ_1 rotated ~20° away from the optimal rotamer. Ile3 → Thr in lysozyme (PDB file 1L18). (**G**) Optimal situation. All database rotamers correspond to the experimentally observed position. Ala98 → Val (PDB file 1L48).

Of the six rules, the first three are the most clear cut, i.e. have a high prediction accuracy. These rules cover ~60% of all cases. In all other cases, the prediction accuracy is lower on average. Reassuringly, however, there is a qualitative correlation between correct prediction and the quality of fit of database fragments on the actual backbone, the number of observed database fragments and the sharpness of the χ_1 distribution of the central residues in these fragments.

Protein structures are highly optimized for functioning in their parent organism. They are not optimized for thermal stability, as can be judged from the relative ease by which proteins can be thermostabilized by mutagenesis (Vriend and Eijsink, 1993) and the marginal stability observed in protein (un-)folding experiments. However, the packing of proteins is optimized in the sense that only few residues are not in an energetically favourable rotameric state. Consequently, core mutations aimed at stabilization of a protein by means of improved packing are normally unsuccessful (Vriend and Eijsink, 1993). It seems very costly to disturb the existing delicate balance of forces in the core of a natural protein. Therefore, a mutated residue appears to adapt to its environment rather than the environment to the mutant. Mutations of a big residue into alanine or glycine rarely lead to major rearrangements in the direct 3-D environment. This supports the hypothesis that most residues, even in closely packed arrangements, are free of torsional strain, and do not need to use the extra space offered to them to relax. Our results strongly suggest that mutations aimed at improving the packing of the core of a protein will only be successful if the mutated residue can be modelled without atomic clashes in its most preferred position-specific rotamer.

Our data set cannot be representative of all engineered point mutations. Despite tremendous progress in all aspects of crystallography over the last couple of years, it still is a major endeavour to solve the structure of a mutated protein. The point mutant structures available in the PDB (Bernstein *et al.*, 1972) are therefore not an ideal set of random mutations, but reflect a process of thought about which mutants are used to answer biologically relevant questions. Mutations that modify the core of a protein and helix capping residues are over-represented in Tables I and II. Also, mutations that lead to isomorphous crystals are probably over-represented; this may imply that the percentage of possible point mutations that lead to a large conformational change is somewhat larger than currently apparent.

Not all conformational changes resulting from point mutations can be predicted correctly with the techniques available to date. Examples of changes difficult to predict are large domain motions or local structure adaptations induced by cofactor binding. However, the surprising ease with which the structure of the vast majority of all mutated residue rotamers can be predicted correctly opens new avenues for planning and analysing protein engineering experiments. Computer screening of large numbers of point mutations becomes feasible, which can save much time and effort otherwise spent on experimental trial and error.

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