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Accuracy of Refined Protein Structures. II. Comparison of Four Independently Refined Models of Human Interleukin 1β

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Abstract

To assess the accuracy of refined structures, a comparison was made using independently determined structures of the same protein in the same crystal form. The models were re-refined against a common data set to minimize the effects of different data and different refinement protocols. The process did not converge to a single model. Rather the structures differed from each other by 0.84 Å which was roughly three times that predicted by a Luzzati analysis [Luzzati (1952). *Acta Cryst.* **5**, 802–810]. The individual structures are equally valid and at least partially independent as evidenced by a reduction of the *R* factor by 0.013 when a simple linear combination is used. Only 29 solvent molecules were common to all four models.

Introduction

Macromolecular crystallographers are often asked how accurate are the structures reported in the literature. To estimate the error, a Luzzati analysis (Luzzati, 1952) is generally carried out that, typically, suggests errors on the order of 0.2–0.4 Å. When a protein can be crystallized in a second space group under the same conditions or if there are multiple copies in the crystallographic asymmetric unit, it is then possible to actually examine the differences. In these cases, aside from random error, the observed differences are generally attributed to crystal packing. The best way to estimate errors would be to redetermine the structure, *i.e.* to re-measure diffraction data and to re-refine the structure from the initial model. This is not practical if the number of atoms is large or if significant modifications of the model, *e.g.* the addition of solvent molecules or large movements of side chains, are required. Inspection of the entries in the most recent release of the Protein Data Bank (Bernstein *et al.*, 1977) shows only three such examples. The structure of chymosin B (rennin) was independently determined by Gilliland, Winborne, Nachman & Wlodawer (1990) and Newman *et al.* (1991). The structure of porcine phospholipase A2 has been independently refined (Dijkstra, Renetseder, Kalk, Hol & Drenth, 1978; Finzel, Ohlendorf, Weber & Salemme, 1991). The structure of human interleukin 1β (h-IL1β) has been independently determined by four groups (Priestle, Schäer & Grütter, 1989; Finzel *et al.*, 1990; Treharne, Ohlendorf, Weber, Wendoloski & Salemme, 1990; Veerapandian *et al.*, 1992). All four groups present data indicating that their model is well refined according to the usual established criteria. The availability of these four coordinate sets in the Protein Data Bank provides a unique opportunity for examining the question of accuracy of reported structures.

IL1β is a member of a family of proteins known as cytokines. These proteins are involved in the mediation of intercellular interactions among cells in the immune system and the inflammatory response (for a review see Mizel, 1989). Since modulation of the immune system would have therapeutic use in treating diseases ranging from arthritis to cancer, there has been a great deal of interest in these molecules. The fact that four structural groups were working on h-IL1β attests to this fact. All four groups succeeded in growing highly ordered crystals from ammonium sulfate solutions at neutral pH. Diffraction data to about 2 Å resolution were collected by diffractometry or with area detectors. The structures were solved using standard multiple isomorphous replacement techniques. The models were built and refined using either *PROLSQ* (Hendrickson & Konnert, 1980) or *RESTRAIN* (Haneef, Moss, Stanford & Borkakoti, 1985). Both these programs minimize a function that is the weighted sum of $(|F_{obs}| - |F_{calc}|)^2$ plus the sum of the squares of deviations of geometric parameters from accepted values.

Chambers & Stroud (1979) made a similar comparison between two independently refined high-resolution structures of inhibited forms of bovine

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trypsin. They found that (1) the r.m.s. differences between C_α's and between all atoms were 0.25 and 0.64 Å, respectively, and (2) each structure generated F_{calc}'s which differed from each other as much as they differed from their F_{obs}'s. A potential problem in generalizing their results arises from the fact that the structures were (1) of the same enzyme but with different inhibitors, (2) from crystals grown under slightly differing conditions, (3) refined to different resolutions and (4) not refined against the same data set. The analysis presented here does not suffer from these shortcomings.

Methods

The coordinates for the refined structures were taken from the Protein Data Bank (PDB; Bernstein *et al.*, 1977). In this analysis, file 1I1B is referred to as Upjohn, file 2I1B is referred to as Ciba, file 4I1B is referred to as CARB, and file 5I1B is referred to as DuPont. First, steps were taken to insure that high r.m.s. values would not be obtained for trivial differences such as ring flips or a translational shift. The space group for h-IL1β is $P4_3$. There are two choices in selecting the unique region of diffraction data to collect. These choices are related by a 180° rotation around the **a** + **b** vector. In Upjohn such an alternate choice was made which required the appropriate rotation of the coordinates. Also since the position the origin along the *c* axis is arbitrary, Upjohn, Ciba and CARB were shifted to be consistent with DuPont. The next step was to examine the side-chain conformations and to flip the aromatic rings or other ambiguous side chains to be consistent with those in DuPont.

To eliminate systematic differences in the four coordinate sets due to different data and refinement protocols, Upjohn, Ciba and CARB were refined against the DuPont diffraction data between 5 and 2.1 Å using *PROLSQ*. Before beginning refinement the slightly different unit-cell dimensions were adjusted to those reported in DuPont through conversion to fractional coordinates. During refinement, the resolution and F/σ limits were systematically varied to alter the energy surface and avoid local minima. Specifically, the resolution was set first to 3.2 Å and $F > 3\sigma$ for four cycles, resolution was then increased to 2.8 Å for two more cycles, to 2.4 Å for two more cycles and to 2.1 Å for two more cycles. For the next two cycles $F > 2\sigma$, for the following two cycles $F > \sigma$, and for the remaining cycles $F > 0$. Tight restraints were placed on the thermal-parameter differences between covalently and hydrogen-bonded pairs of atoms. For the final few cycles the solvent occupancies were allowed to vary in steps of 0.2.

Results and discussion

Comparison before common refinement

The r.m.s. difference among the four coordinate sets before refinement against the same data ranged from 0.22 to 0.39 Å for C_α's and from 0.59 to 1.07 Å for all protein atoms. The most similar coordinates were CARB and Upjohn. The most different C_α coordinates were CARB and DuPont but, considering all protein atoms, the most different coordinates were Ciba and DuPont.

A pair-wise comparison was also made of the F_{calc}'s and α_{calc}'s before refinement. The *R* factors for the F_{calc}'s varied between 0.176 for Ciba and DuPont and 0.231 for DuPont and CARB. These values are comparable to the *R* factors between F_{calc}'s and F_{obs}'s reported by the four groups (see Table 1). A similar result was observed by Chambers & Stroud (1979) in their comparison of inhibited bovine trypsin molecules. The mean absolute phase difference calculated from the models varied between 23° for Ciba and DuPont and 32° for DuPont and CARB. For comparison, the mean absolute phase difference between the solvent-flattened MIR phases to 2.8 Å resolution and the final refined phases for the DuPont refinement was twice this difference at 60°. Taken together these results suggest that there are significant differences in the structures obtained by these four groups.

The major interpretational differences were minor and confined to five regions. The first is the amino terminus. In CARB, DuPont and Upjohn residues 1 and 2 were not included in the refined coordinates. In Ciba these residues are included but with thermal factors above 60 Å^2. The second region is Glu64. In Upjohn this residue, the preceding carbonyl, and following amino N atom are modeled as two distinct conformations with occupancies of 0.6 and 0.4. The higher occupancy conformation agreed best with those in the other three models and was the one used in all subsequent analysis. A third difference occurs around Asn107. This residue is at that end of a loop joining the eighth and ninth β-strands. In Ciba the C_α of this residue is about 2 Å from those of the other three coordinates. The fourth difference is in residues 138–142. This region has the sequence Lys-Gly-Gly-Gln-Asp and displays a great deal of mobility as judged by the refined thermal parameters. Aside from the termini, this segment has the highest *B* values in the entire protein. For this region the CARB and Upjohn conformations are the most similar. In Ciba the carbonyl groups of both glycine residues have been flipped 180°. In DuPont the carbonyl groups of both glycine residues have been rotated about 90° resulting in an alternate position for the Gln141 side chain. The final difference is at the carboxyl terminus. In DuPont the carbonyl

Table 1. *Refinement statistics of IL-1β models – initial*

	Upjohn	Ciba	CARB	DuPont
R factor	0.204	0.198	0.242	0.173
R.m.s. error				
Bond length (Å)	0.025	0.010	0.023	0.020
Bond angles (°)	1.5	2.5	1.4	0.7
Planarity (Å)	0.019	0.010	0.047	0.016
Chiral volume (Å^3)	0.30	0.13	0.38	0.27
Non-bonded contacts (Å)	0.21	0.21	0.22	0.24
ΔB				
Main-chain atoms (Å^2)	1.7	3.0	15.9	0.9
Side-chain atoms (Å^2)	2.6	7.9	17.5	1.2
Hydrogen bonded atoms (Å^2)	5.8	20.0	20.3	3.8
$a = b$ (Å)	54.86	54.90	55.14	54.84
c (Å)	77.02	76.80	76.66	76.75
Data collection	Area detector	Diffractometer	Area detector	Area detector
Refinement program	*PROLSQ*	*PROLSQ*	*RESTRAIN*	*PROLSQ*
Resolution range (Å)	20–2	6–2	?–2	5–2.1
R factor reported	0.189	0.172	0.19	0.173
Atoms in multiple positions	12	0	0	10
Range of thermal factors (Å^2)	16–78	4–122	13–71	15–61
Number of solvents	83	168	91	100
Solvent occupancy	0.2 × *n*	1.0	Free	0.2 × *n*
Mean solvent occupancy	0.70	1.0	0.82	0.82

group of Val151 is flipped so that residues 152 and 153 are placed close to the first β-strand. The other three conformations have these residues in an extended conformation branching away from the amino-terminal β-strand.

Table 1 summarizes the refinement statistics for the four models at the beginning of this analysis. The top line of Table 1 reports the *R* factor of the four coordinate sets against the DuPont diffraction data. These values differ by up to 0.05 from those given in the PDB entries. Undoubtedly, some of these differences are due to differences in structure-factor amplitudes. Some of them may also be due to the varying degrees to which low-resolution data were included in the refinement. The largest *R*-factor differences are observed where the low-resolution boundaries are most different.

An examination of the geometric discrepancies indicates that all four models were refined with similar weights on the geometric restraints. There is, however, quite a different picture when the thermal parameters are examined. In CARB, restraints did not appear to be applied to the thermal parameters. In that model the r.m.s. difference in *B* values between bonded atoms is 18.4 Å^2 compared with 10.5 Å^2 for the next highest case (Ciba). Thermal restraints were the tightest for Upjohn and DuPont where the r.m.s. differences in *B* values between bonded atoms are 3.47 and 2.55 Å^2, respectively.

Another difference among the four models was how solvent molecules were handled. The number of solvent molecules included in the final models varied by a factor of two. In addition, the treatments each group gave to the solvent occupancies were also quite different. In Ciba the occupancies were fixed at 1.0 and the thermal factors were used to absorb any occupancy differences. In CARB the thermal factor and occupancies were both freely refined. In Upjohn and DuPont the occupancies were restrained to multiple of 0.2 with tight thermal restraints. For the three models that refined occupancies, the number of solvent molecules as well as the mean occupancies are comparable.

The number of common solvent molecules, *i.e.* those within 1 Å of each other, ranges from 30 between CARB and Ciba or DuPont to 74 molecules between DuPont and Ciba. For the model with the most solvent molecules, *i.e.* Ciba, less than half were constrained in the other models. Even for the model with the least solvent molecules, *i.e.* Upjohn, less than 2/3 were present in the other models. If only the full occupancy solvent molecules in Upjohn and DuPont are considered, virtually all of them are present in Ciba. However, only half of them are present in CARB. These observations suggest that the solvent molecules included in a refined structure may not be very reliable. Perhaps some solvents are placed into noise peaks in the electron-density maps. Table 2 lists the 29 solvent molecules that are present in all four refined models. All of these molecules are found in the first layer of solvation. Interestingly, not all these solvents are buried. Several have only one ligand and are quite exposed to bulk solvent. In DuPont 11 of the conserved solvent molecules are completely buried as are five non-conserved solvent molecules.

Changes during common refinement

During refinement there were no large-scale alterations in the conformations of any of the models. The r.m.s. movement for all atoms ranged from 0.55 to 0.61 Å. These movements resulted in *R* factors, calculated between the F_{calc}'s obtained before and after refinement, of from 0.10 for Upjohn to 0.20 for CARB. The corresponding mean absolute changes in α_{calc}'s ranged from 11 to 24°. Since the new refinement was against different data and different restraints, changes of this magnitude were reasonable.

Comparison after common refinement

Table 3 shows the refinement statistics after *PROLSQ* refinement as described above. The final *R* factors are comparable to those reported by the groups from their refinements. This suggests that the diffraction data used in this analysis are not atypical.

Table 2. *Solvent molecules common to all four models*

DuPont residue	Hydrogen-bond partner				
201	10 N	40 O	210 O		
202	112 N	112 O	122 O	144 O	
203	80 N	134 N			
204	60 N	60 O	69 O	99 O	
205	97 O	98 O	100 O	114 Oγ	
206	28 O	127 O	130 N	130 O	208 O
208	125 N	130 O	131 O	206 O	
209	47 O	57 O	245* O		
210	10 O	18 N	39 Oε1	201 O	
212	26 N	132 O			
214	71 O	116 Nε2	225 O		
216	123 Oγ	135 O	137 O		
218	32 Oε1	62 O	65 O		
219	54 O	103 O	103 Nζ		
223	20 N	38 O			
225	73 N	214 O			
228	128 N				
229	84 N	84 Oγ			
236	9 Oγ1				
241	126 N	141 O	142 Oδ1		
242	86 N				
245	119 Oδ1	51* Oε1	209* O		
247	93 N				
248	144 N	145 Oδ			
249	98 NεH1				
251	93 N	247 O			
260	97 N				
266	118 N				
269	136 N				

* Denotes a residue in a neighboring molecule.

Table 3. *Refinement statistics of IL-1β models – final*

	Upjohn	Ciba	CARB	DuPont
R factor	0.182	0.172	0.191	0.173
R.m.s. error				
Bond length (Å)	0.021	0.019	0.020	0.020
Bond angles (°)	1.1	0.9	1.0	0.7
Planarity (Å)	0.016	0.014	0.016	0.016
Chiral volume (Å^3)	0.26	0.24	0.27	0.27
Non-bonded contacts (Å)	0.22	0.25	0.22	0.24
ΔB				
Main-chain atoms (Å^2)	1.2	1.3	1.3	0.9
Side-chain atoms (Å^2)	1.6	1.7	1.8	1.2
Hydrogen-bonded atoms (Å^2)	4.6	4.7	4.8	3.8
R.m.s. coordinate change (Å)	0.21	0.28	0.18	—
R.m.s. thermal-factor change (Å^2)	5.3	12.8	2.4	—
R.m.s. occupancy change	0.11	0.29	0.09	—
Range of thermal factors (Å^2)	14 61	16 78	16 67	15 61
Mean solvent occupancy	0.70	0.79	0.74	0.82

The geometries of the final models are nearly always better than those at the start. This is most apparent in the r.m.s. errors of bond angles. The improvement is most likely due to placing a higher weight on geometric restraints in this refinement. In Upjohn and CARB the final *R* factors are higher than that of DuPont while that of Ciba is essentially indentical. It was expected that the final *R* factors would be higher since the DuPont model had been continually refined against these data while the others had not. Obtaining an equivalent *R* factor with Ciba may be a result of having significantly more solvent molecules in the model.

The most striking difference between the numbers in Tables 1 and 3 is the r.m.s. differences in thermal factors between bonded atoms. The original refinement of the CARB model had no apparent restraints on the thermal factors and that of the Ciba model had only weak constraints. The r.m.s. thermal factor change during re-refinement of the Ciba model is over twice that of the next highest. This appears to be the result of a few solvent molecules with extremely high thermal factors initially being restrained to have values similar to those atoms to which they are hydrogen bonded. It is interesting to note that imposing tight geometric and thermal restraints produces *R* factors nearly identical to those reported by the four groups. This suggests that the reduced freedom in parameter space imposed by this refinement protocol does not lead to a significantly higher final *R* factor.

A pair-wise comparison of the F_{calc}'s and α_{calc}'s after refinement was made to see to what extent agreement had improved. The *R* factors between the F_{calc}'s improved only slightly. The range was from 0.160 between CARB and Upjohn to 0.191 between CARB and either Ciba or DuPont. The mean absolute phase differences ranged from 21 to 26° for the same pairs. These numbers should be compared with changes resulting directly from the refinement against the DuPont data (see previous section). If the only differences between the models were the data and refinement protocol used then the models should have converged during refinement to yield much better agreement among the F_{calc}'s and α_{calc}'s.

Since the calculated structure factors did not converge to a single set, it is not surprising that the models also did not converge to the same model. The

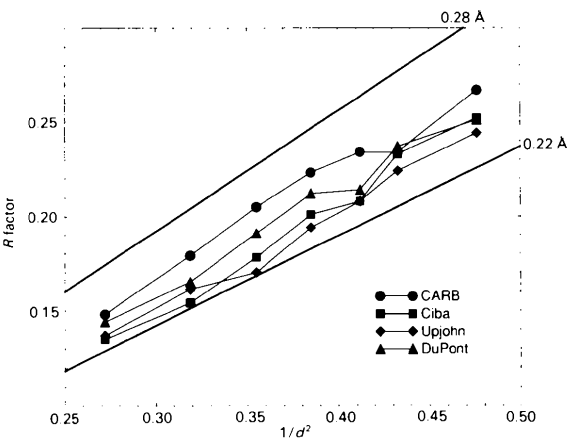

Fig. 1. *R* factors between F_{obs}'s and F_{calc}'s as a function of 1/(resolution)^2 for four models refined against the same diffraction data. Curves show the anticipated error distribution for the mean errors in atomic positions of 0.22 and 0.28 Å (Luzzati, 1952).

r.m.s. coordinate differences between models were not significantly changed from their initial values despite movement during refinement. For C_α's the r.m.s. differences ranged fom 0.22 to 0.39 Å. For all protein atoms the r.m.s. differences ranged from 0.58 to 1.02 Å. Taking all the models together produced an r.m.s. difference of 0.84 Å among all protein atoms. These values can be compared with a Luzzati analysis (see Fig. 1) where the indicated r.m.s. error is of the order of 0.25 Å.

The final question examined was the independence of the final models. That is, do linear combinations of models result in an R factor lower than that observed for any model separately? A search was made using weights ranging from 0.0 to 1.0 in steps of 0.1 for each of the models with the condition that the sum of the weights was unity. The lowest R factor was observed from a model which was one part CARB, two parts Upjohn, three parts Ciba, and four parts DuPont. The R factor from this combination was 0.159. This 0.013 improvement in the R factor suggests that the final models obtained in this analysis are at least partially independent in conformational space and should be regarded as equally valid.

Summary

This analysis of the four independently refined models of h-IL1β has four primary observations.

(1) The mean difference between F_{calc}'s from independent models is of the order of the R factor as reported by Chambers & Stroud (1979).

(2) Different models with similar R factors and geometric quality should be considered as equally valid.

(3) Traditional Luzzati analysis (Luzzati, 1952) produces error estimates which are roughly a third of that actually found between independently refined models.

(4) The positions of solvent molecules are the least reliable part of a structural model and may be the result of over-interpretation.

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