Journal of Protein Chemistry, Vol. 13, No. 7, 1994

A Comparison of the Predicted and X-Ray Structures of Angiogenin. Implications for Further Studies of Model Building of Homologous Proteins

Simon C. Allen,[1] K. Ravi Acharya,[1,5] Kathleen A. Palmer,[2] Robert Shapiro,[3,4] Bert L. Vallee,[3] and Harold A. Scheraga[2]

Received May 10, 1994

The three-dimensional structure of human angiogenin has been determined by X-ray crystallography and is compared here with an earlier model which predicted its structure, based on the homology of angiogenin with bovine pancreatic ribonuclease A. Comparison of the predicted model and crystal structure shows that the active-site histidine residues and the core of the angiogenin molecule, including most of the β-strands and α-helices, were predicted reasonably well. However, the structure of the surface loop regions and residues near the truncated C-terminus differs significantly. The C-terminal segment includes the active-site residues Asp-116, Gln-117, and Ser-118; Gln-117 in particular has been shown to be important in affecting the ribonucleolytic activity of angiogenin. Also, the orientation of one helix in the model differed from the orientation observed experimentally by about 20°, resulting in a large displacement of this chain segment. The difficulty encountered in predicting the surface loop regions has led to a new algorithm [Palmer and Scheraga (1991), *J. Comput. Chem.*, **12,** 505–526; (1992), *J. Comput. Chem.*, **13,** 329–350] for predicting the conformations of surface loops.

KEY WORDS: X-ray crystallography; computer modeling; angiogenin; ribonuclease A.

1. INTRODUCTION

A significant number of protein structures determined by X-ray crystallographic and NMR techniques have been demonstrated to fall into structural families, in which some or all of the tertiary structures are similar. Examples include the dehydrogenases, having a dinucleotide-binding (Rossmann) fold (Rossmann *et al.*, 1975), proteins with an α/β barrel (Farber and Petsko, 1990), the immunoglobulin family (Williams and Barclay, 1988), and lysozyme and α-lactalbumin (Acharya *et al.*, 1990). It has also been observed that three-dimensional structures are conserved to a far greater degree than the corresponding amino acid sequences (Rossmann *et al.*, 1974; Blundell *et al.*, 1987; Chothia and Lesk, 1987). On this basis, a number of putative three-dimensional structures have been predicted for proteins where an X-ray structure of a homologous protein can serve as a starting model (Browne *et al.*, 1969). Typically, the sequences have been at least 35% identical, with active-site residues largely conserved, many non-identical residues substituted conservatively, and deletions and insertions occurring primarily in the surface loops of flexible regions.

Using these principles of homology modeling,

[1] School of Biology and Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, UK.
[2] Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853-1301.
[3] Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, Massachusetts 02115.
[4] Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.
[5] To whom correspondance should be addressed.

0277-8033/94/1000-0641$07.00/0

```
ANG Struc   ---HHHHHHHH  HHHH------  HHHH.HHHHHH  HHH-----.--  EEEEEEE-HH
ANG Model   ..---HHHHHH  HH--------  ----.-HHHHH  HH------.--  --EEEE----
5RSA Struc  ..---HHHHHH  HHH-------  -----HHHHHH  HH---------  -EEEEE---H

ANG         MQDNSRYTHFL  TQHYDAKPQG  RDDR.YCESIM  RRRGLTSP.CK  DINTFIHGNK
RNase A     ..KETAAAKFE  RQHMDSSTSA  ASSSNYCNQMM  KSRNLTKDRCK  PVNTFVHESL

ANG seq.           10          20          30          40          50
number

ANG Struc   HHHHHHHH--  -EEEE--..-EE  EEE--EEEEE  EEEE------  --EEEEEEE
ANG Model   ---------E  E------..-EE  E----EEEEE  EE--------  ----EEEEE
5RSA Struc  HHHH-----E  EE--------EE  E----EEEEE  EEE-------  ---EEEEEE

ANG         RSIKAICENK  NGNPHRE..NLR  ISKSSFQVTT  CKLHGGSPWP  PCQYRATAGF
RNase A     ADVQAVCSQK  NVACKNGQTNCY  QSYSTMSITD  CRETGSSKYP  NCAYKTTQAN

ANG seq.           60          70          80          90         100
number

ANG Struc   E-EEEEEE--  ..EEEEEE3333  3--
ANG Model   E--EEE----  ..----EEE--.  ...
5RSA Struc  E-EEEEEE--  --EEEEEEEE-.  ...

ANG         RNVVVACENG  ..LPVHLDQSIF  RRP
RNase A     KHIIVACEGN  PYVPVHFDASV.  ...

ANG seq.          110         120
number
```

Fig. 1. Sequence alignment and structure classification for ANG and RNase A. The numbering with respect to ANG is recorded. ANG, Angiogenin amino acid sequence; RNase A, bovine pancreatic ribonuclease A amino acid sequence. Structure classification: ·, not in this structure/sequence; -, neither a helix nor a strand. H, Helix; E, strand; 3, 3_{10} helix; ANG Struc, angiogenin structure (Acharya *et al.*, 1994); ANG Model, predicted angiogenin model (Palmer *et al.*, 1986); 5RSA Struc, ribonuclease A structure (5RSA coordinates from Brookhaven protein data bank).

a preliminary model of human angiogenin (ANG)[6] was constructed (Palmer *et al.*, 1986) based on the structure of bovine pancreatic ribonuclease A (RNase A) (Wlodawer *et al.*, 1982). Using the X-ray structure of RNase A, the backbone and side chains of the ANG molecule were fitted taking the amino acid deletions into consideration. The resultant model was subjected to conformational energy minimization with a penalty distance function constraining the backbone structure to that of RNase A, allowing the backbone and side-chain dihedral angles to vary. In the final stages of energy minimization, the penalty distance function was removed. In all, the model constructed by Palmer *et al.* (1986) for human ANG had a conformation closely similar to that of RNase A.

Angiogenin was first isolated in 1985 based on its capacity to induce angiogenesis, the formation of new blood vessels (Fett *et al.*, 1985). The protein was derived from a human adenocarcinoma cell line and its amino acid sequence revealed a significant homology to the pancreatic ribonucleases (Strydom *et al.*, 1985); the amino acid sequence of ANG is 33% identical to that of RNase A. The aligned sequences are illustrated in Fig. 1. Three residues critical for ribonucleolytic activity in RNase A, His-12, Lys-41, and His-119 (Richards and Wyckoff, 1973), have counterparts in the ANG sequence. RNase A has four disulfide bonds, three of which are equivalent in ANG: the disulfide bond between Cys-65 and Cys-72 in RNase A has no counterpart in the ANG sequence. There are six deletions in the ANG sequence as compared to RNase A, an extra residue at the N-terminus, and four additional residues at the C-terminus. The extra residues at the termini were not incorporated into the model because they seemed likely to project out into the solvent and would not affect the structure of the core of the molecule.

The ribonucleolytic activity of angiogenin is at

[6] Abbreviations: ANG, Human angiogenin; ASH, Analyze Structural Homology; DSSP, Display Secondary Structure of Proteins; ECEPP, Empirical Conformational Energy Program for Peptides; RNase A, bovine pancreatic ribonuclease A.

a much lower level than that of RNase A (Shapiro *et al.,* 1986; Lee and Vallee, 1989), and RNase A does not exhibit angiogenic activity. The objective of the construction of a structural model based on RNase A was the exploration of the origins of these functional differences in terms of sequence differences. Moreover, such a model would provide a basis for the design of site-directed mutagenesis experiments to understand the function of the ANG molecule.

The crystal structure of ANG has recently been determined to a resolution of 2.4 Å (Acharya *et al.,* 1994). Here, we compare the X-ray structure with the model constructed by Palmer and colleagues and evaluate the validity of the model.

2. RESULTS AND DISCUSSION

Figure 2a–c shows stereo drawings of the crystal structures of ANG, the predicted model, and the crystal structure of RNase A. Structure superpositions were carried out with two programs, SHP (Structure Homology Program; D. I. Stuart, unpublished results), based on the method of Rossmann and Argos (1976); and ASH (Analyze Structural Homology; D. I. Stuart, unpublished results), based on the algorithm of Hendrickson (1979). Superimposing the ANG crystal structure and the predicted model using the two programs produces almost exactly the same result, but SHP bases the comparison on spatially equivalent residues, whereas ASH compares sequentially equivalent residues. The program ASH was used for the overall superpositions, except where stated. The rms deviations for selected segments of structure were also calculated using ASH, considering only those selected residues for the superposition calculation, to give a better estimation of the modeling in that particular region.

The rms deviations for all, main-chain, and C^{α} atoms between the crystal structure and the predicted model and between the crystal structures of ANG and RNase A are shown in Tables IA and IB, respectively. The rms deviation for C^{α} atoms alone is 3.2 Å for the 118 residues in both the model and the structure; the rms deviation between all 943 atoms is 4.4 Å. SHP, comparing 111 spatially equivalent residues, gives an overall deviation of 2.20 Å for C^{α} atoms. Figure 3 shows the rms deviations for individual residues in the model (Palmer *et al.,* 1986) and the X-ray structure (Acharya *et al.,* 1994). The rms deviation between all atoms in the crystal structures of ANG and RNase A is 6.5 Å; SHP, comparing 116 spatially equivalent residues, gives an overall deviation of 2.26 Å for C^{α} atoms.

Secondary structure elements of ANG (Acharya *et al.,* 1994), the predicted ANG model, and RNase A are shown in Figs 1 and 2. In general, these elements have been predicted well in the model. Both the model and the ANG X-ray structure contain seven β-strands. Only two of these differ markedly in position: strand 2, which lies within an extended loop that encompasses residues 59–68, and strand 7, near the C-terminus. Of the three helices in the ANG crystal structure, helix 1 was predicted accurately and helix 2 was predicted at an angle of 20° to the helix in the structure. The residues for helix 3 were predicted in an approximate helical conformation, but their dihedral angles ϕ, ψ and hydrogen bonding pattern are such that the program DSSP [Display Secondary Structure of Proteins (Kabsch and Sander, 1983)] does not classify them as helical.

Regions of interest and regions where the structure and the model differ in conformation are discussed below.

2.1. Residues 1–5

After optimal superposition of this *local* segment, the rms deviation between coordinate sets for all atoms in residues 2–5 of ANG and the model is 2.02 Å.

The residues Met-(−1) and Gln-1 in the crystal structure of ANG were not incorporated into the model. The native protein sequence has a blocked N-terminus and its first residue is pyroglutamate; the preceding methionine in the recombinant protein does not affect the biological function of the protein (Shapiro *et al.,* 1988). The first residue in both the structure and the model is Asp-2, corresponding to Lys-1 in the RNase A sequence. The first ten residues of the ANG structure and their counterparts in the ANG model are shown in Fig. 4. Clearly, the helix starting at Ser-4 corresponds well, but the first two residues of the model have very different conformations from those in the structure. The main-chain atoms of Asp-2 and Asn-3 are 4–5 Å away from the equivalent residues in the model. They form a loose turn, defined as a bend by DSSP, which is not predicted by the model. The first three or four residues in the structure do have a relatively high

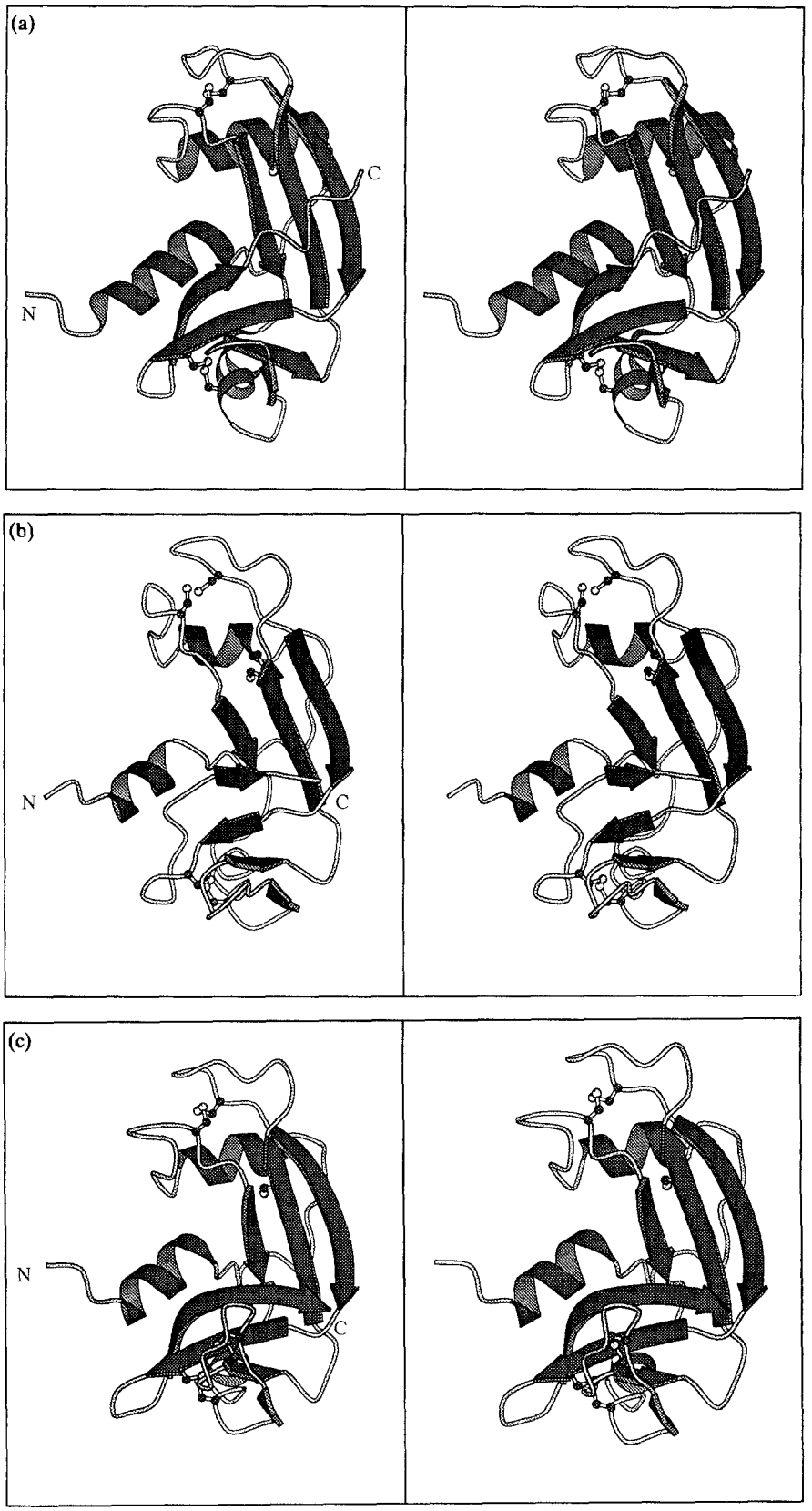

Fig. 2. Stereo diagrams, with helices represented as coils and strands as arrows. The side chains of cystine residues are shown, and the N- and C-termini are indicated. (a) The crystal structure of ANG. (b) The predicted model of ANG, based on the structure of RNase A. (c) The crystal structure of RNase A. The representations of the structures in this and subsequent figures are drawn with the program MOLSCRIPT (Kraulis, 1991).

Table IA. RMS Deviations Between the Crystal Structure of ANG (Acharya *et al.*, 1994) and the Predicted Model (Palmer *et al.*, 1986), from the Program ASH

Residue numbers	All atoms	Main-chain atoms	α Carbons
2–119	4.44 Å	3.15 Å	3.21 Å

Table IB. RMS Deviations Between the Crystal Structures of ANG (Acharya *et al.*, 1994) and RNase A (Wlodawer *et al.*, 1982), from the Program ASH

Residue numbers	All atoms	Main-chain atoms	α Carbons
1–124	6.48 Å	6.04 Å	6.16 Å

degree of flexibility as assessed by both the quality of the electron density maps and their temperature (B) factors, but there is little suggestion that they adopt the conformation observed in the model.

It should be noted that it is generally difficult to model the N- and C-termini. These regions tend to have greater inherent flexibility and solvent exposure than the rest of the molecule. Perhaps more significantly, as close atomic contacts are inevitably introduced during the course of modeling, the structure shifts in response to the repulsive forces. In the modeling, the termini of the chain are free to move to relieve these close contacts, and are therefore less likely to attain the optimum packing arrangement corresponding to the X-ray structure.

2.2. Residues 15–22

After optimal superposition of this *local* segment, the rms deviation between coordinate sets for all atoms in residues 15–22 of ANG and the model is 2.81 Å.

This region covers a long segment of extended chain between helix 1 and helix 2. The conformations of the X-ray structure and the ANG model are very different, largely because of the wrong assignment of the dihedral angles for proline-18. The proline side chain points toward the interior of the molecule in the structure, but toward the

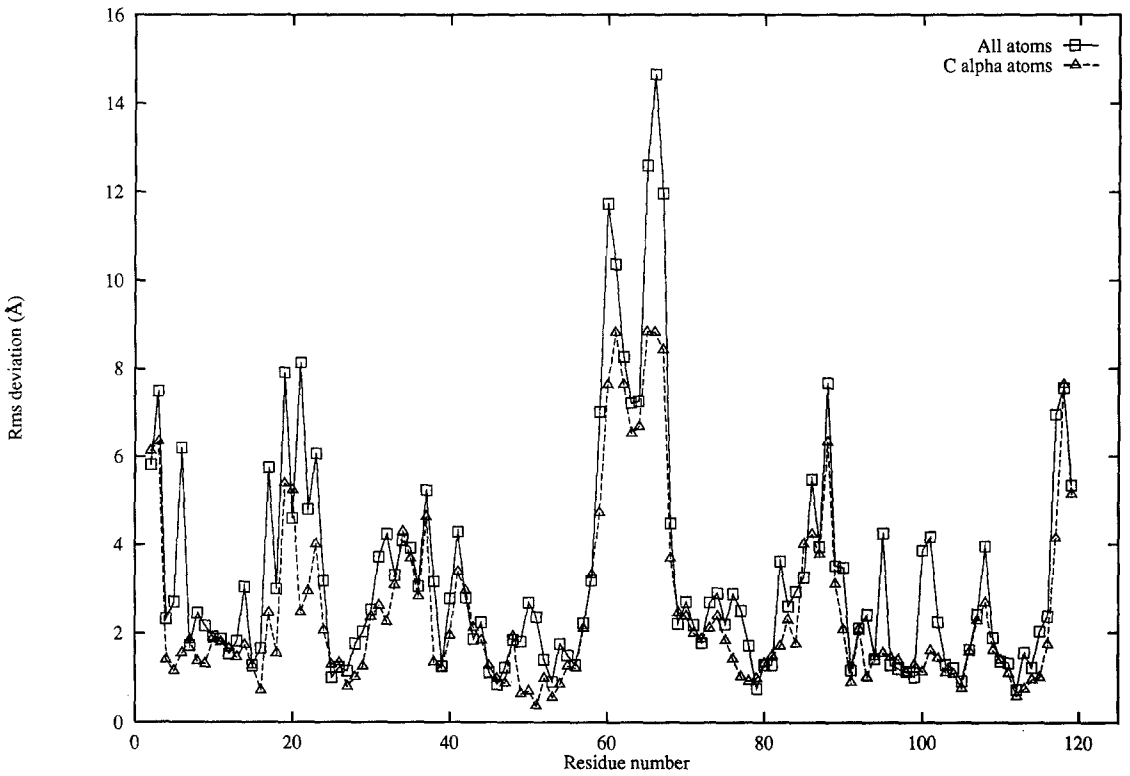

Fig. 3. The rms differences for all atoms (continuous line) and α carbons (broken line) between the ANG crystal structure (Acharya *et al.*, 1994) and the predicted model (Palmer *et al.*, 1986). The comparison was done using the program ASH.

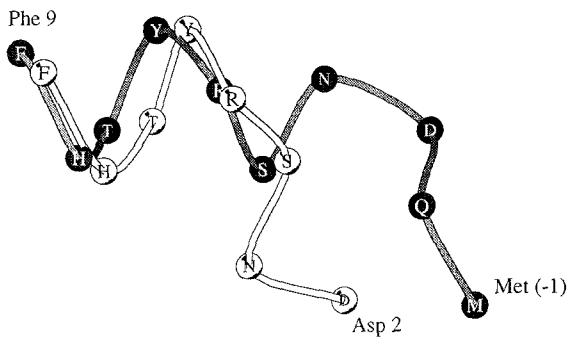

Fig. 4. An α-carbon trace of residues −1 to 9 of the ANG crystal structure (gray) and residues 2–9 of the predicted model (white). Phe-9 and the first residues of the structure, Met-(−1) and the model, Asp-2, are indicated.

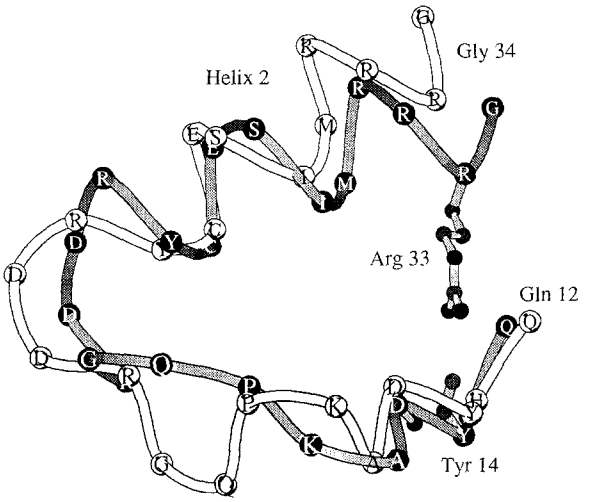

Fig. 5. Residues 12–34. The figure shows helix-2 (residues 21–33) and residue 34 of the ANG crystal structure (gray) and corresponding residues from the predicted model (white), packing against residues 12–20. The side chain of Arg-33 and the peptide and carbonyl of Tyr-14 are shown for the ANG crystal structure.

exterior in the model. As a consequence, the subsequent residues are more external in the structure. This contributes to the large rms deviation for these residues.

In subsequent modeling studies, some problems with the geometry and energy of the proline residue in the ECEPP (Empirical Conformational Energy Program for Peptides) algorithm (Momany *et al.*, 1975, Némethy *et al.*, 1983) were noted. These parameters were then revised (Némethy *et al.*, 1992), taking into account structural information that became available after the ECEPP parameters were first developed. The new parameters for proline have proven to be more satisfactory, and it is reasonable to surmise that problems in modeling proline were due, in part, to the inadequate parameters for the proline residue itself in the earlier ECEPP algorithm.

2.3. Residues 21–33, Helix 2

After optimal superposition of this *local* segment, the rms deviation between coordinate sets for all atoms in residues 21–23 of ANG and the model is 3.46 Å.

The first residue in the model indentified as helical by DSSP is Cys-26, where the agreement between the crystal structure and the model is good; the position of the disulfide bond is retained. Residues 21–25 in the model superimpose less well with the equivalent residues in the structure, where there is a stronger hydrogen bonding pattern, clearly forming an α-helix; in the model, the necessary hydrogen bonds are not formed. For example, in the structure, a hydrogen bond is formed between the carbonyl oxygen of Asp-22 and the main-chain nitrogen of the half-cystine, at position 26, which are 3.10 Å apart. In the model, the distance between these two atoms is 7.29 Å. Similarly, in the structure, a hydrogen bond is formed between the carbonyl of Asp-23 and the main-chain nitrogen of Glu-26, 3.13 Å away. In the model, these are 6.88 Å apart. The relatively good agreement for Cys-26 continues for Glu-27 and to a lesser extent for Ser-28, but after this there is an increasing difference as the helix progresses. This arises from the fact that the angle between the helix and the rest of the structure is different in the crystal structure and the model (Fig. 5). In the crystal structure, the helix packs more closely against the rest of the protein, i.e., the region of extended chain between residues Tyr-14 and Gly-20 and the β-sheet comprising β-1 (residues 41–47), β-4 (76–84), and β-5 (93–101). In the model, the helix leans away from the rest of the tertiary structure and is therefore more exterior. The angle between the helices in the structure and the model is approximately 20°, which is reflected in the increasing difference in the C^{α} positions following the sequence away from the disulfide bond between Cys-26 and Cys-81. This results in the positions of the side chains being different, although their overall orientation is the same. An example of the sort of effect that this difference causes is observed

in the case of Arg-33. In the crystal structure, there are hydrogen bonds 2.93 and 2.95 Å long between the nitrogens of the Arg-33 guanidinium group and the carbonyl oxygen of Tyr-14 (the conformation of this side chain and the carbonyl group is illustrated in Fig. 5), whereas in the model, the nitrogens of this group are 4.42 and 6.55 Å away from the carbonyl oxygen. The hydrogen bond between the Arg-33 guanidinium group and the carbonyl group of Thr-11 is found in both the model and the structure. Similar differences in main-chain position, but not side-chain orientation, continue until Pro-38, with the structure and the model coming to a closer agreement for the disulfide bond connecting Cys-39 to Cys-92.

2.4. Residues 57–69

After optimal superposition of this *local* segment, the rms deviation between coordinate sets for all atoms in residues 57–69 of ANG and the model is 6.15 Å.

Over this region of the protein, the coordinates of the crystal structure and the predicted model are radically different, as shown in Fig. 6. The disulfide bond between Cys-57 and Cys-107 is similar in both, but after this the conformation differ. In the crystal structure, residues Gly-62 to His-65 form a short β-strand on the surface of the molecule with loops at both ends. In the predicted model, Lys-60 and Asn-61 have a β conformation, but not residues 62–65. This segment of the protein is important because residues 60–68 have been proposed as a receptor binding sequence, critical for the angiogenic activity of ANG (Hallahan *et al.*, 1991, 1992). Therefore, the conformation of this region has particular significance in the examination of its functional role, especially in the design of mutagenesis experiments. These residues are among the least conserved between the amino acid sequences of RNase A and ANG (Bond *et al.*, 1993) and, with respect to RNase A, two residues are deleted in the human ANG sequence, between Glu-67 and Asn-68. Also, this is the segment of the protein where RNase A has an extra disulfide bond, not present in ANG. The cysteines are replaced by Pro-64 and Leu-69 in ANG.

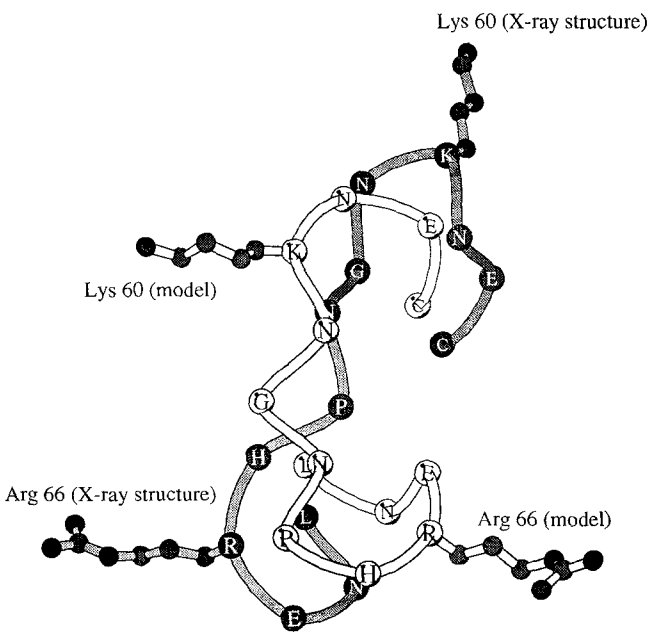

Fig. 6. Residues 57–69. An α-carbon trace of the conformation of the loop in the X-ray structure (gray) and the model (white). The side chains for Lys-60 and Arg-66 are indicated.

2.5. Residues 85–91

After optimal superposition of this *local* segment, the rms deviation between coordinates sets for all atoms in residues 85–91 of ANG and the model is 2.98 Å.

The conformations in the X-ray structure and in the predicted model deviate from one another in this loop quite significantly. These residues, particularly Trp-89, have been implicated in the binding of ANG to placental ribonuclease inhibitor, a protein that inhibits both the enzymatic and angiogenic activity of ANG (Shapiro and Vallee, 1987; Lee and Vallee, 1989; Lee *et al.*, 1989). A part of the sequence of this loop region between strands β-4 (residues 76–84) and β-5 (93–101) is unusual: Gly-Gly-Ser-Pro-Trp-Pro-Pro. The flexible nature of glycines and the *cis*/*trans* conformers for proline mean that several different conformations would be energetically plausible. Hence, it is not surprising that the predicted model varies from the crystal structure. Palmer *et al.* (1986) noted that the characteristics of this loop made it difficult to model and decided to keep Pro-90 (equivalent to Pro-93 in RNase A) in the *cis* conformation that it adopts in the RNase A structure, but to assume that the other prolines in the loop (indeed all the other prolines in the model) should be in a *trans* conformation. In the ANG X-ray structure, Pro-91 has a *cis* conformation and the other prolines are *trans*.

2.6. Residues 115–123

After optimal superposition of this *local* segment, the rms deviation between coordinate sets for all atoms in residues 115–119 of ANG and the model is 3.85 Å.

Residues after Ile-119 were not included in the predicted model for ANG. Leu-115 shows good agreement between the structure and the model, but the remaining residues do not have the same conformation as in the crystal structure. In the structure, Gln-117 to Arg-121 form a short 3_{10} helix, which has no parallel in the model. One of the reasons for the poor prediction is this region may be that residues 120–123 were not included in the model: although these residues were hypothesized to project away from the core of the protein, in fact they form main-chain hydrogen bonds with the preceding residues, and the side chains of Phe-120 and Arg-121 are quite close to the active site (Acharya *et al.*, 1994).

2.7. The Ribonucleolytic Active Site

The essential residues His-13 and His-114 are predicted well and are in the same conformation in both the structure and the model (Fig. 7). They also superimpose closely with the RNase A structure. Thr-44, residue thought to participate in pyrimidine binding (Curran *et al.*, 1993), is also in the same conformation. However, the model does not predict the positions of the other active-site residues so well. Lys-40 is on a loop which has been modeled slightly inaccurately: its main-chain atoms are approximately 2 Å away from their counterparts in the X-ray structure and its side chain has been modeled pointing away from, rather than toward, the catalytic site (Fig. 7). The most significant difference is that, in the X-ray structure, Gln-117 adopts a conformation that obstructs the site corresponding to the pyrimidine-binding pocket of RNase A: this can be seen clearly in Fig. 7, where the glutamine residue side chain passes through the

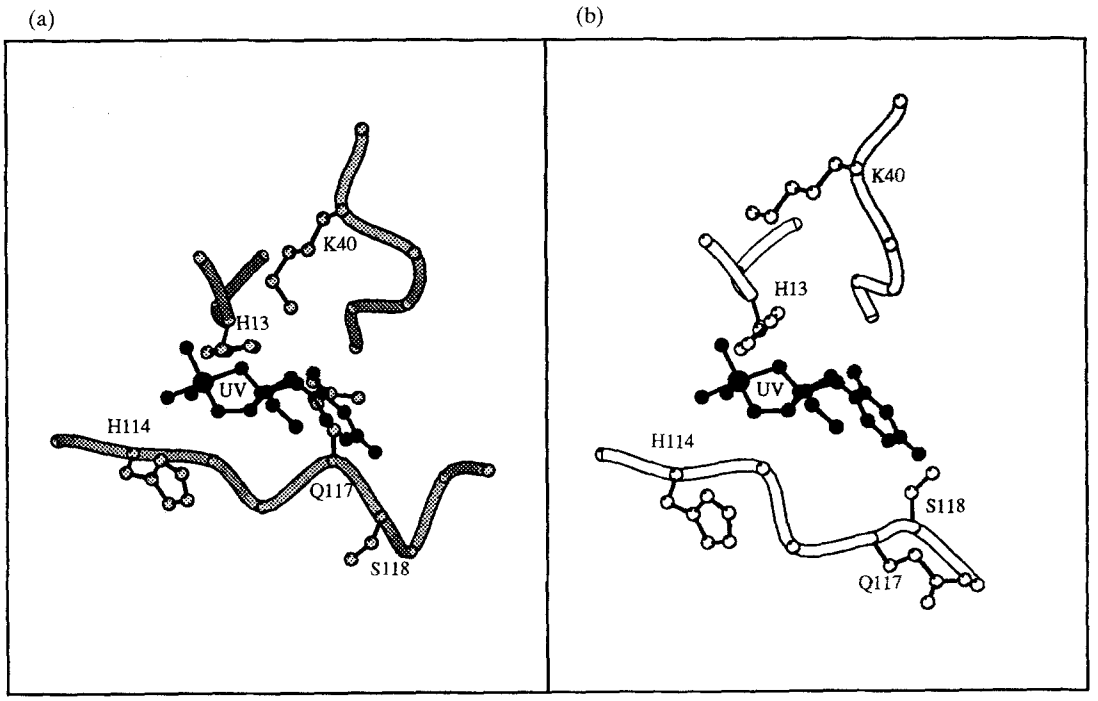

Fig. 7. The active-site region of modeled complexes of human ANG with uridine vanadate (UV). (a) The X-ray structure, in gray. (b) The model, in white. The coordinates for the complex of UV with RNase A (Borah *et al.*, 1985) were obtained from the Brookhaven Protein Data Bank (6RSA). The ANG X-ray structure was superimposed on the RNase A structure using the program ASH, and the ANG model was superimposed on the ANG structure. The side chains of His-13, Lys-40, His-114, Gln-117, and Ser-118 are shown. An α-carbon trace is shown for residues 12–15, 39–44, and 113–119. UV is shown in black.

uracil ring of the inhibitor uridine vanadate, modeled into the active site (Acharya *et al.*, 1994). The amide group of the side chain is about 9 Å away from its position in the model. The X-ray structure studies (Acharya *et al.*, 1994) and recent mutagenesis studies (Russo *et al.*, 1994) show that the conformation of this residue is probably one of the most significant sources of the lower ribonucleolytic activity seen in ANG as compared to RNase A. The adjacent residue, Ser-118, is thought to form part of the pyrimidine-binding site in RNase A (as Ser-123), but in ANG, mutation of this residue fails to alter specificity (Curran *et al.*, 1993). In the model, this residue is in a markedly different conformation from its counterpart in the ANG crystal structure (Fig. 7). In the X-ray structure, the residue points away from the active site; the α carbons of Ser-118 in the structure and in the model are 7.6 Å away and the side-chain oxygen atoms 8.5 Å away. It should be noted, however, that mutagenesis results (Russo *et al.*, 1994) suggest that the C-terminal region of ANG must undergo a rearrangement in order for RNA to be cleaved. It is possible that the 'active' conformation adopted after this reorientation is more similar to that seen in the predicted ANG model.

3. CONCLUSIONS

A significant number of models have been predicted for unknown protein structures, based on known crystal structures (e.g., Warme *et al.*, 1974; Swenson *et al.*, 1978; Blundell *et al.*, 1983, 1987; Weber *et al.*, 1989). The basis for building models—that structural conservation is more pronounced than amino acid sequence conservation and that a number of protein 'families' are becoming apparent—has been noted above.

However, the literature contains considerably fewer comparisons between models and subsequently determined three-dimensional structures (e.g., Acharya *et al.*, 1990; Teeter *et al.*, 1990; Weber, 1990), and models have met with varying degrees of success.

In the present comparison, the essential three-dimensional structure of the core of ANG was predicted reasonably well, with the exception of helix 2. The angle between the helix in the structure and that in the model is quite significant and results in differences of up to 7 Å between the two superimposed coordinate sets. Therefore, the tertirary structure predicted by the model is incorrect for the interactions between this helix and the rest of the protein.

The other discrepancies between the X-ray structure and the predicted model occur mainly in the surface loop regions. Most importantly, residues 60–68, which have been implicated in receptor binding, are very different. Some atoms are more than 18 Å away from the positions of their modeled counterparts. There are considerable differences between the RNase A and ANG sequences over this region and the absence of a disulfide bond would be expected to lead to a different conformation. However, the model by Palmer *et al.* (1986) does not predict the correct structure. Similar cases occur for residues 17–23 and 85–91, where the sequences are different from the corresponding residues in RNase A and the structure and predicted model vary considerably.

It would seem, then, that despite the overall similarity between the ANG and RNase A amino acid sequences, the sequence similarity between the surface loops in particular was not significant enough for accurate template-based three-dimensional structure prediction. In their original paper, Palmer *et al.* (1986) recognized the necessity of divising methods to model surface loops correctly, and subsequently developed an analytical procedure for such a purpose (Palmer and Scheraga, 1991, 1992). With this procedure, they were able to model surface loop regions in lysozyme and ribonuclease. As a consequence, this newer procedure should increase the reliability of model building by homology. Therefore, the deviations reported here should be reduced by applying this newer procedure to ANG.

ACKNOWLEDGMENTS

We thank Drs. David Phillips, Anthony Rees, and James Riordan for their support and encouragement. This work was supported by the Medical Research Council and the Leverhulme Trust U.K., through grants to K.R.A., and the Endowment for Research in Human Biology, Boston, Massachusetts (R.S. and B.L.V.). The work at Cornell University was supported by grants from NSF (DMB90-15815) and NIH (GM-14312); support was also received from the National Foundation for Cancer Research.

REFERENCES

Acharya, K. R., Stuart, D. I., Phillips, D. C., and Scheraga, H. A. (1990). *J. Protein Chem.* **9,** 549–563.

Acharya, K. R., Shapiro, R., Allen, S. C., Riordan, J. F., and Vallee, B. L. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 2915–2919.

Blundell, T. L., Sibanda, B. L., and Pearl, L. (1983). *Nature* **304,** 273–275.

Blundell, T. L., Sibanda, B. L., Sternberg, M. J. E., and Thornton, J. M. (1987). *Nature* **326,** 347–352.

Bond, M. D., Strydom, D. J., and Vallee, B. L. (1993). *Biochim. Biophys. Acta* **1162,** 177–186.

Borah, B., Chen, C. W., Egan, W., Miller, M., Wlodawer, A., and Cohen, J. S. (1985). *Biochemistry* **24,** 2058–2067.

Browne, W. J., North, A. C. T., Phillips, D. C., Brew, K., Vanaman, T. C., and Hill, R. L. (1969). *J. Mol. Biol.* **42,** 65–86.

Chothia, C., and Lesk, A. M. (1987). In *Cold Spring Harbor Laboratory Symposium on Quantitative Biology,* Vol. 52, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 399–405.

Curran, T. P., Shapiro, R., and Riordan, J. F. (1993). *Biochemistry* **32,** 2307–2313.

Farber, G. K., and Petsko, G. A. (1990). *Trends Biochem. Sci.* **15,** 228–234.

Fett, J. W., Strydom, D. J., Lobb, R. R., Alderman, E. M., Bethune, J. L., Riordan, J. F., and Vallee, B. L. (1985). *Biochemistry* **24,** 5480–5486.

Hallahan, T. W., Shapiro, R., and Vallee, B. L. (1991). *Proc. Natl. Acad. Sci. USA* **88,** 2222–2226.

Hallahan, T. W., Shapiro, R., Strydom, D. J., and Vallee, B. L. (1992). *Biochemistry* **31,** 8022–8029.

Hendrickson, W. A. (1979). *Acta Crystallogr. A* **35,** 158–163.

Kabsch, W., and Sander, C. (1983). *Biopolymers* **22,** 2577–2637.

Kraulis, P. J. (1991). *J. Appl. Crystallogr.* **24,** 946–950.

Lee, F. S., and Vallee, B. L. (1989). *Biochem. Biophys. Res. Commun.* **161,** 121–126.

Lee, F. S., Auld, D. S., and Vallee, B. L. (1989). *Biochemistry* **28,** 219–224.

Momany, F. A., McGuire, R. F., Burgess, A. W., and Scheraga, H. A. (1975). *J. Phys. Chem.* **79,** 2361–2381.

Némethy, G., Pottle, M. S., and Scheraga, H. A. (1983). *J. Phys. Chem.* **87,** 1883–1887.

Némethy, G., Gibson, K. D., Palmer, K. A., Yoon, C., Paterlini, G., Zagari, A., Rumsey, S., and Scheraga, H. A. (1992). *J. Phys. Chem.* **96,** 6472–6484.

Palmer, K. A., and Scheraga, H. A. (1991). *J. Comput. Chem.* **12,** 505–526.

Palmer, K. A., and Scheraga, H. A. (1992). *J. Comput. Chem.* **13,** 329–350.

Palmer, K. A., Scheraga, H. A., Riordan, J. F., and Vallee, B. L. (1986). *Proc. Natl. Acad. Sci. USA* **83,** 1965–1969.

Richards, F. M., and Wyckoff, H. W. (1973). In *Atlas of Molecular Structures in Biology. 1. Ribonuclease S* (Phillips, D. C., and Richards, F. M., eds.), Clarendon, Oxford.

Rossmann, M. G., and Argos, P. (1976). *J. Mol. Biol.* **105,** 75–95.

Rossmann, M. G., Moras, D., and Olsen, K. W. (1974). *Nature* **250,** 194–199.

Rossmann, M. G., Liljas, A. Brändén, C.-I., and Banaszak, L. J. (1975). In *The Enzymes,* Vol. 11, Academic Press, New York, pp. 61–102.

Russo, N., Shapiro, R., Acharya, K. R., Riordan, J. F., and Vallee, B. L. (1994). *Proc. Natl. Acad. Sci. USA* **91,** 2920–2924.

Shapiro, R., and Vallee, B. L. (1987). *Proc. Natl. Acad. Sci. USA* **84,** 2238–2241.

Shapiro, R., Riordan, J. F., and Vallee, B. L. (1986). *Biochemistry* **25,** 3527–3532.

Shapiro, R., Harper, J. W., Fox, E. A., Jansen, H.-W., Hein, F., and Uhlmann, E. (1988). *Anal. Biochem.* **175,** 450–461.

Strydom, D. J., Fett, J. W., Lobb, R. R., Alderman, E. M., Bethune, J. L., Riordan, J. F., and Vallee, B. L. (1985). *Biochemistry* **24,** 5486–5494.

Swenson, M. K., Burgess, A. W., and Scheraga, H. A. (1978). In *Frontiers in Physico-chemical Biology* (Pullman, B. ed), Academic Press, New York, pp. 115–142.

Teeter, M. M., Ma, X. Q., Rao, U., and Whitlow, M. (1990). *Proteins: Structure Function Gene.* **8,** 118–132.

Warme, P. K., Momany, F. A., Rumball, S. V., Tuttle, R. W., and Scheraga, H. A. (1974). *Biochemistry* **13,** 768–782.

Weber, I. T. (1990). *Proteins: Structure, Function and Gene.* **7,** 172–184.

Weber, I. T., Miller, M., Jaskolski, M., Leis, J., Skalka, A. M., and Wlodawer, A. (1989). *Science* **243,** 928–931.

Williams, A. F., and Barclay, A. N. (1988). *Annu. Rev. Immunol.* **6,** 381–405.

Wlodawer, A., Bott, R., and Sjölin, L. (1982). *J. Biol. Chem.* **257,** 1325–1332.