Crystal Structures of an Archaeal Class I CCA-Adding Enzyme and Its Nucleotide Complexes

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Summary

CCA-adding enzymes catalyze the addition of CCA onto the 3’ terminus of immature tRNAs without using a nucleic acid template and have been divided into two classes based on their amino acid sequences. We have determined the crystal structures of a class I CCA-adding enzyme from Archaeoglobus fulgidus (AfCCA) and its complexes with ATP, CTP, or UTP. Although it and the class II bacterial Bacillus stearothermophilus CCA enzyme (BstCCA) have similar dimensions and domain architectures (head, neck, body, and tail), only the polymerase domain is structurally homologous. Moreover, the relative orientation of the head domain with respect to the body and tail domains, which appear likely to bind tRNA, differs significantly between the two enzyme classes. Unlike the class II BstCCA, this enzyme binds nucleotides non-specifically in the absence of bound tRNA. The shape and electrostatic charge distribution of the AfCCA enzyme suggests a model for tRNA binding that accounts for the phosphates that are protected from chemical modification by tRNA binding to AfCCA. The structures of the AfCCA enzyme and the eukaryotic poly(A) polymerase are very similar, implying a close evolutionary relationship between them.

Introduction

Mature tRNA molecules contain a universally conserved 3’-terminal CCA sequence. This sequence plays a crucial role in protein biosynthesis, since it is the site of tRNA aminoacylation (Sprinzl and Cramer, 1979) and interacts with the large subunit of the ribosome to enable peptide bond formation (Green and Noller, 1997; Nissen et al., 2000). Indispensable as it is, however, the CCA sequence is not encoded in many eubacterial and archaeal tRNA genes and nearly all eukaryotic tRNA genes (Aebi et al., 1990). The maturation of tRNA therefore requires an essential polymerase, the CCA-adding enzyme (tRNA nucleotidyltransferase) that catalyzes the posttranscriptional addition of the CCA sequence using CTP and ATP as substrates (Deutscher, 1982). In those few eubacterial species that, like E. coli, encode this CCA triplet in their tRNA genes, the CCA-adding enzyme serves to rebuild the CCA ends of tRNAs that have been degraded by exonucleolytic attacks (Zhu and Deutscher, 1987).

In general, polynucleotide polymerases require a template strand that specifies the sequence of nucleotides to be incorporated, with the notable exceptions known to date of the poly(A) polymerase (PAP), the terminal deoxynucleotidyltransferase (TdT), and the CCA-adding enzyme, all of which function without a nucleic acid template. Among these template-independent polymerases, the CCA-adding enzyme is the most intriguing since its nucleotide incorporation has the highest degree of sequence and length specificity. Also remarkable is the occurrence of two classes of CCA-adding enzymes that do not show homologous regions outside of the catalytic domain (Li et al., 2002), suggesting that this enzyme activity might have arisen twice in evolution.

The CCA-adding enzyme belongs to the nucleotidyltransferase superfamily (NT) that is defined by an active site sequence of G[SG]LIVMVFyR[GQ]x5,D[LIVM][DE]-[CLIVMFY]x2 (Holm and Sander, 1995; Yue et al., 1996). The NT family includes a strikingly diverse array of enzymes that add nucleotides to DNA, RNA, protein, and antibiotics (Holm and Sander, 1995), and has been divided into two classes based on their sequences (Yue et al., 1996). The sequences of class I enzymes have little similarity to other enzymes within the class or to the class II enzymes outside the signature motif in the catalytic domain, whereas class II enzymes share a homologous 25 kDa N-terminal region but differ in their C-terminal domains. Examples of Class I enzymes include archaeal CCA-adding enzymes, DNA polymerase β (pol β), eukaryotic PAP, TdT, and kanamycin nucleotidyltransferases (KNT). Class II enzymes include eubacterial and eukaryotic CCA-adding enzymes and eubacterial PAPs (Yue et al., 1996; Martin and Keller, 1996).

Crystal structures are known for the class I enzymes, pol β, KNT, TdT, and eukaryotic PAP, and for the class II enzymes, eubacterial, and eukaryotic CCA-adding enzymes (Pelletier et al., 1994; Sakon et al., 1993; Delarue et al., 2002; Bard et al., 2000; Martin et al., 2000; Li et al., 2002; Augustin et al., 2003). These structures demonstrate that all NT family members share a homologous polymerase domain (Li et al., 2002), and contain additional domains that have different structures and functions.

Over the past several decades a large body of information has been obtained to illuminate the enzymatic process of the CCA-adding enzyme. The current understanding of the enzymatic reaction can be summarized as follows. (1) The nucleotide addition is catalyzed by a two-metal ion mechanism that is the same for all polymerases (Steitz, 1998); (2) the enzyme recognizes primarily the acceptor stem and the TuC stem-loop of the tRNA molecule (Shi et al., 1998); (3) the tRNA molecule does not move with respect to the enzyme during the
course of CCA addition (Shi et al., 1998); and (4) there is a single catalytic domain that contains sequence homologous to the catalytic site of DNA pol \( \beta \) (Holm and Sander, 1995; Yue et al., 1998), and a single nucleotide binding pocket exists for both CTP and ATP, which are recognized by a “protein template” on the enzyme (Li et al., 2002). Crystal structures have been obtained for two class II CCA-adding enzymes from the eubacterium Bacillus stearothermophilus (BstCCA) and human (hmtCCA) (Li et al., 2002; Augustin et al., 2003). The structure of the hmtCCA is almost identical to that of BstCCA, except that its C-terminal domain was disordered and not observed in the crystal structure. In the structures of the BstCCA binary complexes, the incoming ATP and CTP are specifically recognized by an arginine and an aspartate from the neck domain (Li et al., 2002).

We have determined the crystal structures of a class I CCA-adding enzyme from Archeoglobus fulgidus (AfCCA), a 51 kDa molecular weight protein containing 437 amino acids, and its nucleotide complexes with ATP, CTP, or UTP. This class I enzyme has similar overall dimensions and analogous subdomains to the class II bacterium enzyme BstCCA. However, their structural homology is limited to the head (or polymerase) domain only. The orientation of the body and tail domains relative to the head domain differs in the two enzymes. Because the body and tail domains are likely to be involved in tRNA binding, it appears that the acceptor stem of the tRNA is presented to the active site differently in the two enzymes, raising the possibility that the mechanism of specificity for CCA addition is accomplished differently. Also, in contrast to BstCCA, the AfCCA enzyme binds nucleotides nonspecifically in the absence of tRNA. Interestingly, the structure of this enzyme is homologous throughout most of its length to another class I enzyme, the eukaryotic poly(A) polymerase, providing structural evidence for the suggestion that they have a close evolutionary relationship and catalytic mechanisms (Yue et al., 1996).

Results and Discussion

The Structure of the Class I Archaeal CCA-Adding Enzyme

The crystal structures of apo AfCCA and its complexes with ATP, CTP, or UTP have been determined at resolutions between 2.1 Å and 1.8 Å. The apo structure was initially solved by multiple isomorphous replacement methods in a crystal form with space group C222. This structure was subsequently used to determine by molecular replacement the structure of a second crystal form with space group C2, which was then refined (Table 1). The structures of the nucleotide complexes were determined using unbiased Fo-Fc difference Fourier methods before the NTP structures were included in the model. All four structures have good geometry with free R-factors between 21.6% and 23.6% (Table 1).

The AfCCA structure consists of four domains named the head, neck, body, and tail (Figure 1), analogous to those observed in the class II BstCCA (Li et al., 2002). The domain architecture of AfCCA exhibits an elongated cleft, with the head and tail domains forming the end walls and the body domain lining the bottom (Figure 1B). The head domain has the five-stranded \( \beta \) sheet flanked by \( \alpha \) helices that is found in all members of the NT family. The neck domain contains mixed \( \beta \) sheets and \( \alpha \) helices that are homologous to the central domain of the eukaryotic PAP (DALI Z-score of 9.8) (Holm and Sander, 1998). The body domain, which is composed of a four-stranded \( \beta \) sheet flanked by \( \alpha \) helices, is connected to the neck through a flexible loop and makes hydrophobic contacts with the neck via a long central helix (Figure 1B). This domain is topologically similar to the RNA binding domains of the RNA-recognition motif (RRM) protein family that includes more than 200 members (Varani and Nagai, 1998). The tail domain of AfCCA extends over 25 Å from the body and appears to be loosely attached to the rest of the enzyme. This tail conformation is stabilized by dimer interactions with a symmetry related molecule in the unit cell (Figure 1C).

### Comparison between the Class II CCA-Adding Enzymes and the Class I AICCA and Poly (A) Polymerases

Although both the AICCA and BstCCA enzymes have similar dimensions and numbers of domains, their structural homology is limited to the head domain, which contains the three catalytically essential carboxylates, Glu59, Asp61, and Asp110, that bind divalent metal ions (Figure 2). The head domains of these two enzymes superimpose with an rmsd of 1.7 Å between 50 corresponding C\(_{\alpha}\) atoms. Interestingly, the structures of palm domains of AICCA and either rat or human pol \( \beta \) are more similar to each other than those of the two classes of CCA-adding enzymes. The rmsd for superposition of equivalent C\(_{\alpha}\) atoms is 1.8 Å for the former pair. There are two additional \( \beta \) strands in the class II BstCCA palm that are not present in this structure.

The neck, body, and tail domains of AICCA contain numerous \( \beta \) strands, in contrast to the exclusively \( \alpha \)-helical domains in the class II BstCCA. In addition, the orientation of the long axis of the elongated body and tail domains with respect to the head domain differs by nearly 90° between the two classes of CCA-adding enzymes (Figures 1B and 1E). As discussed below, the dimensions of the cleft and its surface electrostatic potential distribution suggest that the body and tail domains may be involved in tRNA binding. The difference in the orientations of the putative tRNA binding domains implies a difference in the direction of the tRNA acceptor stem helix with respect to the polymerization active site.

The AICCA molecule dimerizes via its body and tail domains, whereas the class II BstCCA dimerizes using a smaller interface surface that is formed by its head domains (Figure 1F). As a result, the catalytic domains are at the two far ends in the class I dimer, whereas the two catalytic domains are in close proximity at the center of the class II dimer.
Comparison of the AfCCA structure with that of another class I enzyme, the eukaryotic poly(A) polymerase, reveals a striking resemblance between them (Figures 1A and 1D). A search for proteins whose structures are similar to the AfCCA structure using the DALI program gives a Z score of 18.0 for the PAP structure (Bard et al., 2000; Martin et al., 2000). In fact, when AfCCA is superimposed on PAP, the head and neck domains differ by an rmsd of 2 Å for 170 corresponding C atoms, while the body domain superimposes with an rmsd of 1.9 Å for 42 C atoms. These two different enzymes have the same fold throughout their entire polypeptide chain, excluding the AfCCA tail domain, which was not present in the structure of the truncated PAP. However, the orientation of the body domain relative to the neck domain differs by a rotation of about 30° between these two enzymes. It has been observed in vitro that PAP can add poly A tails of several hundred nucleotides to tRNA (Raynal et al., 1998) and overexpression of PAP in an E. coli mutant strain can partially compensate for the absence of CCA-adding enzymes (Reuven et al., 1997). On the other hand, CCA-adding enzymes have been shown to have polycl synthesis activity, which is reminiscent of the polycl synthesis activity by PAP (Seth et al., 2002). Consistent with these functional data, a comparison of the AfCCA and the class I eukaryotic PAP structures provides a structural basis for the suggestion that there might have been an interconversion of CCA-adding and polycl polymerase activities early in evolution (Yue et al., 1986).

### Apo-AfCCA Recognizes the Triphosphate and Sugar Moieties of NTP
The apo-AfCCA does not recognize the base of an incoming NTP and consequently is unable to discriminate between correct and incorrect NTPs. We have determined the structures of complexes between AfCCA and ATP, CTP, and UTP that were prepared by soaking the nucleotides into the crystals (the binding of GTP was not studied in this work). The first two NTPs are substrates for the enzyme, but UTP is not. For all three nucleotides, the electron density is well defined for the triphosphate and the sugar moieties. Although there is clearly some electron density for the adenosine of ATP, in all three cases the base is not well ordered (Figure 3), presumably as a consequence of the lack of base-specific interactions. The triphosphate group makes extensive hydrogen-bonding interactions with residues in the head and neck domains. His133, which is hydrogen-bonded to the O2′ hydroxyl of the ribose, is probably responsible for distinguishing the incoming NTPs from dNTPs. Since the nonsubstrate UTP binds, this enzyme is not nucleotide specific in the absence of the tRNA substrate. In contrast, the class II BstCCA apo-enzyme is capable of discriminating against UTP or GTP (Li et al., 2002).

Three metal ion binding sites (A, B, and C) are seen located near the catalytic carboxylates in an anomalous difference Fourier map calculated using data from crystals of the UTP complex in which Mn2+ ions have been substituted for Mg2+ ions by soaking experiments. When the catalytic domains of AfCCA and DNA pol β are superimposed, two of the three metal ions superimpose on metal ions A and B in the ternary complex of DNA pol β (Pelletier et al., 1994). The third metal ion is located between the third catalytic carboxylate Asp110 and His101 on an adjacent β strand (Figure 3). Metal ions A and C are also observed in the apo-AfCCA structure without a nucleotide substrate. Interestingly, although the side chains of the residues that chelate the metal ions adopt essentially the same conformation in all three nucleotide complexes, the electron density for metal ion A is very weak in the AfCCA-CTP complex, and clear density can only be observed for metal ion B in the AfCCA-ATP complex.

### A Model of tRNA Bound to the Body and Tail Domains
A model of tRNA bound to the AfCCA enzyme has been built that accounts for numerous biochemical and structural observations (Figure 4A). Several lines of evidence suggest that the body and tail domains of CCA-adding enzymes bind tRNA. First, truncation of the tail domain of the E. coli class II enzyme abolishes the addition of both A and C (Zhu and Deutscher, 1987). Second, the body domain of AfCCA has an RRM-like fold that also occurs in proteins that bind in duplex RNA, for example, the ribosomal protein S6 and phenylalanyl-tRNA synthetase (Lindahl et al., 1994; Zheltonosova et al., 1994). Third, the extended cleft of the AfCCA enzyme is about 60 Å wide and could provide a snug fit for the tRNA acceptor stem and TψC stem-loop (Figure 4A). Interestingly, the class II BstCCA structure has a cleft of similar

### Table 1. Phasing and Refinement Statistics

<table>
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<tr>
<th>Phasing</th>
<th>Native</th>
<th>Au</th>
<th>Hg</th>
<th>Pt</th>
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<td>9.5%</td>
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<td>71.9%</td>
<td>96%</td>
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<tr>
<td>Phasing power (centric/accentric)</td>
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<td>1.0/1.2</td>
<td>0.3/0.4</td>
<td></td>
</tr>
<tr>
<td>Figure of merit (2.8 Å)</td>
<td>0.56 (MIR); 0.67 (after density modification)</td>
<td></td>
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### Refinement

| Space group | C2 (a = 86.15 Å, b = 79.95 Å, c = 78.14 Å, β = 97.53) |
| Rsym       | 8.1%   | 6.5% | 6.8% | 5.2% |
| Completeness| 99.2%  | 99.7%| 99.9%| 97.9%|
| Rwork/Rfree | 19.1%/23.6% | 18.4%/22.8% | 18.7%/22.6% | 18.3%/21.6% |
shape (Figure 4B), suggesting that its body and tail domains may also have a tRNA binding role. Fourth, and perhaps most compellingly, the surface electrostatic potential of AfCCA exhibits a strip of positive electrostatic potential on the body and tail domains, which is consistent with its being the site of tRNA binding. A tRNA substrate was modeled onto the AfCCA enzyme to accommodate the charge and shape complementarities (Figure 4A). The acceptor and T stems of tRNA were placed into the extended cleft of the enzyme, with the CCA terminus in the active site and the T loop contacting the tail domain, and the anticodon stem and loop was pointed away from the enzyme. In this model the tRNA contacts only one AfCCA subunit of the dimer and a second tRNA could bind to the other monomer without steric clash, consistent with native gel shift experiments that show a molar ratio of 1:1 for the AfCCA-tRNA complex (see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/12/5/1165/DC1).

This model is strongly supported by biochemical protection and interference experiments. Phosphates of the acceptor and T stems of the tRNA are protected from alkylation by ethylnitrosourea when the tRNA is bound to the AfCCA enzyme (Shi et al., 1998). Further, alkylation of these phosphates and a few additional ones interfere with the CCA-adding activity (Shi et al., 1998). These
The polymerase domain is shown in magenta and the neck/fingers in green. The incoming nucleotide is yellow and the metal ions are brown. The three catalytic carboxylates are shown. In (B), the last magenta helix (cylinder) to the right belongs to Pol β fingers domain. For comparison with the AfCCA head it is colored the same as the palm domain. In (C), the region colored in red represents the additional part of BstCCA not seen in the AfCCA head and Pol β domains.

phosphates lie largely on one side of the tRNA helices and are mostly in contact with the enzyme in the modeled complex (Figure 4A). The loss of activity due to alkylation of the two phosphates in the variable loop could be attributed to an alteration of the structure of tRNA by the chemical modification, rather than arising from interference of the direct interaction of these phosphates with the enzyme (Figure 4A). Furthermore, this model explains (1) the specificity of the CCA-adding enzyme for tRNA since its shape is complementary only to tRNA or tRNA-like structures, and (2) the nonprocessivity of tRNA during the CCA addition since the tail domain would block its translocation after each nucleotide addition. In spite of its different structure, a similar model for tRNA binding can also be constructed for the class II enzymes in a manner that accommodates the shape and charge complementarity, although the surface charge distribution is not as positive (Figure 4B).

Implications for Nucleotide Selection

The binding of nucleotides to the class I AfCCA enzyme appears to be nonspecific in the absence of the tRNA substrate. In contrast, ATP or CTP bound to the active site of the class II BstCCA is specifically recognized by an arginine (R157) and an aspartate (D154) from the neck domain. Although we cannot rule out the possibility that the crystal lattice restricts the enzyme from undergoing a conformational change that confers nucleotide selectivity, this seems unlikely. Despite the fact that *Archeoglobus fulgidus* is a thermophile, AfCCA has been shown to have significant activity (>50%) at a lower temperature of 20°C (H.-D. Cho and A.M.W., unpublished data), indicating that the crystals we obtained at room temperature contain enzyme molecules that can assume an active conformation. More likely, the nucleotide complexes that we observe in the crystal structures reflect a nonselective property of the apo-enzyme. The capacity of the enzyme to select the correct incoming nucleotide might require some direct interaction between the base and the acceptor stem, as suggested earlier (Shi et al., 1998). Alternatively, binding of the tRNA substrate...
Figure 4. Models of tRNA Bound to Monomers of the Two Classes of CCA-Adding Enzymes that Account for tRNA Protection Experiments

(A) The AfCCA enzyme is shown in a surface representation and colored blue for negative electrostatic potential and red for positive electrostatic potential. tRNA is shown in stick representation with the backbone path highlighted by a pink coil. The magenta spheres indicate those phosphates that are protected from alkylation by tRNA binding to the enzyme and/or those that interfere with activity when alkylated. The orange balls indicate phosphates whose alkylation interferes with CCA-adding activity. (B) A model of the BstCCA complex with tRNA that has been color coded the same as in (A).

could produce a conformational change in the active site pocket that is required to position protein side chains for specific base interactions. Regardless of which pathway is used by the enzyme, it appears that the tRNA substrate is required for nucleotide selection, in contrast to the class II apo enzyme that appears able to provide a "template" for the incoming nucleotide.

Implications for the CCA-Adding Process and Its Evolution

The domain architectures of both classes of CCA-adding enzymes exemplify a recurring theme in polymerase structures: the core polymerase domain (the palm) is conserved and probably the ancestral form of the superfamily. Subsequent evolution of separate family members resulted in the acquisition of additional domains that possess distinct enzymatic or regulatory activities (Joyce and Steitz, 1995; Aravind and Koonin, 1999), which in this case includes the binding of tRNA and the base specificity for the incoming nucleotide. Intriguingly, the putative tRNA binding domains (body and tail) in the two classes of CCA-adding enzyme are not oriented in the same way relative to the catalytic site (Figure 5). This implies that the direction from which the primer strand extending from the tRNA acceptor stem approaches the active site differs in the two enzymes. As a consequence the single stranded primer terminus cannot be continuously stacked on the acceptor stem in both enzymes and still have the primer terminus bind to the catalytic site in the same way as presumably it must be. It appears that the 3′ terminal nucleotide is not stacked on the acceptor stem in class I enzymes.

Taken together with their differences in the nucleotide selection, this appears to reinforce the speculation that the CCA-adding enzymes evolved independently twice (Aravind and Koonin, 1999).

Since a CCA-adding activity presumably resided in the last common ancestor of the three domains of life, it is puzzling to consider why eubacteria and eukaryotes have class II CCA-adding enzymes, while the archaea have the very different class I enzyme. It has been suggested (Aravind and Koonin, 1999) that the eukaryotes acquired its class II CCA enzyme by lateral transfer from eubacteria. But what form of enzyme provided the CCA-adding activity prior to that, and why are the archaean enzymes so different? Perhaps the CCA-adding activity of the last common ancestor was provided by either a ribozyme or more likely a ribonuclear protein consisting of the common catalytic domain augmented by RNA domains to bind tRNA and select nucleotides. If, then, a complete protein replacement of the RNA component evolved subsequent to the divergence of the three domains of life, the present occurrence of two classes of CCA-adding enzymes could be explained. An alternative hypothesis would posit that the CCA trinucleotide was encoded by the tRNA genes even after the three domains of life diverged and that the CCA-adding enzymes evolved subsequently as evolving RNases made repair activity essential. This would not, however, explain why the otherwise very different enzymes of the two classes share a common NT polymerase catalytic domain.

Conclusion

Comparison of the crystal structure of a class I CCA-adding enzyme, AfCCA, with that of the class II enzyme,
Crystal Structure of Class I-Adding Enzyme

Experimental Procedures

Crystallization and Data Collection

The AfCCA molecule containing a his-tag at its C terminus was expressed and purified as described previously (Yue et al., 1998). The final concentration of the enzyme was adjusted to 6 mg/ml before crystallization. Two crystal forms were found. Crystal form 1 belongs to space group C222₁ and grew from a solution containing 100 mM MES (pH 6.2), 5 mM MgCl₂, 400 mM NaCl, and 40% ethanol. The crystals were cryoprotected with paratone-N oil and flash frozen in liquid propane. 2.4 Å resolution diffraction data from the native crystal and heavy atom soaks were collected at various synchrotron beamlines including X25 at the National Synchrotron Light Source (NSLS; Upton, NY), ID19 and ID14 at the Advanced Photon Source (APS; Argonne, IL), and A1 and F2 at the Cornell High Energy Synchrotron Source (CHESS, Ithaca, NY). A second crystal form was obtained in space group C2, after 200 mM CaCl₂ was added to the starting crystallization mixture. Crystals complexed with NTPs were obtained by soaking the apo crystals in mother liquor containing 4 mM ATP, CTP, or UTP and the MgCl₂ was replaced by 5 mM MnCl₂. Data from the second crystal form were collected at beamlines X26C at NSLS and ID19 at APS. All data were processed using the HKL package (Chwinowski and Minor, 1997), and the statistics are summarized in Table 1.

Structure Determination and Refinement

The apo-AfCCA structure was solved in the C2₂₂₁ crystal form by multiple isomorphous replacement methods using salts of the heavy atoms Au, Hg, Pt, and U. There is one monomer per asymmetric unit. The program SOLVE (Terwilliger and Berendzen, 1999) was used to locate the positions of the heavy atoms and to calculate single isomorphous replacement (SIR) phases using the Au derivative data set. The heavy atom sites with lower occupancy that occurred in other heavy atom derivatives were found by difference Fourier methods (CCP4 package) using the SIR phases, which yielded excellent starting experimental phases. After density modification, very clear electron density could be seen for the neck, body, and tail domains. However, the head domain was not well ordered and only the backbone C atoms could be built. At this point the second crystal form in space group C2 was available, and a molecular replacement solution was obtained using a partial model built in the C₂₂₂₁ crystal form. In the C2 crystal form the head domain is ordered and multi-crystal electron density averaging between the two crystal forms using the program Dmmulti (Cowtan, 1994) greatly improved the electron density in this region. The final model was built with the graphics program O (Jones et al., 1991) and refined with the program Refmac5 (Murshudov et al., 1997) to obtain free R-factors between 21.6% and 23.6%. The current model includes 437 protein residues, (two molecules of NTP in each of the nucleotide complexes, respectively.

Acknowledgments

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References


Figure 5. A Comparison of the Different Orientations that the tRNA Acceptor Stem and T/C Stem-Loop Have with Respect to the Head Domains in the Two Classes of CCA-Adding Enzyme (A) AfCCA. (B) BstCCA. The head domains of the two enzymes are represented in magenta ribbons and are in the same orientation. The enzymes are shown with surface representation and the acceptor stem and T/C stem-loop of the tRNA in yellow coil.


Yue, D., Maizels, N., and Weiner, A.M. (1996). CCA-adding enzymes and poly(A) polymerases are all members of the same nucleotidyltransferase superfamily: characterization of the CCA-adding enzyme from the archaeal hyperthermophile Sulfolobus shibatae. RNA 2, 895–908.


Accession Numbers

The coordinates and diffraction amplitudes have been deposited in the Protein Data Bank with accession numbers 1R8A, 1R8B, 1R89, and 1R8C.

Note Added in Proof

The structure of the AFCCA enzyme derived from the same crystal form as the present study has just been published (EMBO J. 22, 5918–5927); however, these authors’ conclusions concerning the binding of ATP and CTP as well as the complex with 1RNA are significantly different from our results. Okabe et al. have interpreted their electron density for the incoming nucleotide differently, placing the base in the position of the ribose, the ribose in the position of the alpha-phosphate, and the gamma-phosphate in the place of water. This results in models for the triphosphate moieties that differ significantly from other ones, have unusual geometry, and have metal ion interactions that differ from all other polymerases, including the homologous DNA pol beta. Further, they constructed a complex with 1RNA that has one 1RNA per dimer that is rotated by 90 degrees from the 1:1 complex model presented here. Their model is not consistent with the biochemical 1RNA protection and interference data (Shi et al., 1998) and our data showing a 1:1 protein subunit to 1RNA complex (see Supplemental Figure S1).