Structure determination of ribosomal protein L2 by multiplewavlength anomalous diffraction method

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INTRODUCTION

In all living cells, protein synthesis is carried out in cellular organelles called ribosomes. These large ribonucleoprotein complexes are generally organized in two subunits of unequal size. In prokaryotic *Escherichia coli* ribosomes, the large (50S) subunit consists of 34 proteins and 5S and 23S rRNAs, while the small (30S) subunit is a complex of 21 proteins and 16S rRNA. The fundamental activity of the ribosome is to decode the message of the mRNA and to form a peptide bond between peptidyl-tRNA and aminoacyl-tRNA (peptidyl transferase). The protein L2 is known to be most important constituent of the peptidyl transferase center. Furthermore, the protein L2 is a primary 23S rRNA-binding protein and is known to play a crucial role in assembly of the domain IV in 23S rRNA. We have crystallized the recombinant protein corresponding to the central region of the L2 (60 - 201, *Bst*L2-RBD), which has been shown to be a 23S rRNA-binding domain. We tried to collect diffraction data on the beamline 41XU at the SPring-8, however, no good diffraction data was obtained due to the lack of good L2 crystals during our beamtime. Nonetheless, we have succeeded in solving its three-dimensional structure, which we report here.

RESULTS

Diffraction data was collected at BL18B at the Photon Factory. MAD diffraction data of recombinant selenomethionyl *Bst* L2-RBD were collected in cold nitrogen gas stream at 100 K. Three data sets were collected on and around the selenium K absorption edge. Data were integrated using DENZO. Scaling and processing were performed using the CCP4 program suite.

The Bijvoet and dispersive anomalous difference Patterson maps were solved by the SHELXS-97. Heavy-atom parameter refinement and phase calculations were carried out using the program SHARP, and the initial electron-density map was subsequently improved by real-space solvent flipping with SOLOMON. The model was refined against the "Remote" data with the program X-PLOR. The refined model has an R-factor of 19.9% for 91% of the data between 20Å and 2.3 Å, including 63-194 and 60-194 residues for crystallographically independent molecules, respectively, and 57 water molecules, for a total of 2075 atoms. The free R-factor for the remaining 9% of the data within this resolution range is 28.6%. The rms deviation from standard values of bond lengths and bond angles are 0.007 Å and 1.276°, respectively.

The figure shows a stereo view of the overall structure of *Bst* L2-RDB. The molecule has an all beta structure consisting of two domains of approximately the same size. The amino terminal domain has a five-stranded β-sheet and is folded into an open β-barrel structure with its open side facing the carboxyl terminal domain. The carboxyl terminal domain is also folded into a five-stranded β-barrel. The C-terminal β-barrel is characterized by a pair of short antiparallel β-sheets facing each other. These two domains are connected by a short 3_{10} helix and are arranged so to create a putative RNA-binding site between them. The barrel axes of the two domains are approximately perpendicular to each other.