aureus and shown that it differs from that described for E. coli (4,5). Moreover, we are also analysing Dsb systems in bacteria containing an extended array of Dsb proteins and results from this work also suggest divergent redox mechanisms. This research is not only providing a comprehensive picture of the process of oxidative protein folding in vivo, but also, given the role of Dsb proteins in the pathogenicity of microbes, the investigated proteins represent putative targets for the development of antimicrobials with a novel mechanism of action.

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Keywords: enzyme structure determination, protein crystallography, bacterial pathogenesis

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Crystal structure of Dxp reductoisomerase from *Geobacillus stearothermophilus*

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Isopentenyl diphosphate (IPP) is an essential compound for living organisms as a precursor of isoprenoids, such as hormones, cholesterols and carotenoids. Mammals use the mevalonic acid pathway, on the other hand, many eubacteria, plastid and malaria parasites use the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. Inhibitors of the MEP pathway, therefore, are considered as effective antibacterial, antimalarial drugs and herbicides, which are harmless to human. In this pathway, we have focused on 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), which is responsible for the second step in the pathway. To characterize this enzyme, several DXR structures from *Escherichia coli*, *Zymomonas mobilis* and *Mycobacterium tubercrosis* have been reported with or without cofactors/substrate/inhibitors. The primary sequences of DXRs from those bacteria are highly homologous, however, based on the comparison of those crystal structures, we could observe the differences in the binding manner of inhibitors in the active sites. In order to analyze the inhibition mechanism further, we have started to study the DXR structure from *Geobacillus stearothermophilus*. We have successfully obtained crystals by the hanging drop vapor diffusion method with NADPH and Mg^{2+} for cocrystallization. We originally tried to solve the structure by the molecular replacement method without success, thus we performed the Se-SAD method to determine the phase using the program SHARP. The structure of GsDXR was refined at 1.9 Å resolution. The overall structure of GsDXR shows no significant differences with those of other DXRs. The electron density of the flexible loop region covering the active site was not observed clearly, and we are trying to obtain the complex structure with the inhibitor.

Keywords: MEP pathway, antimalarial drug, SAD

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Crystal structure of the thermostable mutant of hygromycin phosphotransferase from *Escherichia coli*

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Aminoglycoside antibiotics, such as hygromycin, kanamycin, neomycin, spectinomycin, and streptomycin, inhibit protein synthesis by acting on bacterial and eukaryotic ribosomes. These antibiotics are widely used for selection of transformants in molecular biology with the combination of the corresponding resistant genes. These selection markers, however, had been available at normal temperature except one for kanamycin/neomycin. We have recently obtained the thermostable mutant of hygromycin B phosphotransferase (Hph) (EC 2.7.1.119) from *Escherichia coli* by the directed evolution method. This mutant (Hph5) increased its thermostablity at 16 ℃ compared to the wild type and can be used as a selection marker for *Thermus thermophilus*. Hph from *E. coli* converts hygromycin B to 7 "-*O*-phosphohygromycin using the phosphate moiety from ATP, resulting in the loss of its cell-killing activity. In order to analyze the mechanism of its catalytic activity and thermostablity, we have crystallized the Hph5 protein for the first time by the hanging-drop vapour diffusion method. The crystal provides diffraction data to a resolution of 2.1 Å and belongs to space group $P3_221$ with unit-cell parameters $a = b = 71.0$, $c = 125.0$ Å. We also obtained the crystal complexes of Hph with hygromycin B and AMP-PNP or ADP in the same crystal form as that of the apoprotein. The structure was composed of N-terminal β-sheet domain and C-terminal α-helix domain, which is similar to that of protein kinases. Base on the comparison of apo and holo structures, Hph does not seem to show a conformational change according to the substrate binding or modification, which is typical in case of protein kinases.

Keywords: aminoglycoside antibiotics, kinase, thermostability

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Crystal structures of N^5-CAIR synthetase (PurK) from *A. aeolicus*, *T. thermophilus* and *S. tokodaii*

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The 6th reaction in the purine nucleotide biosynthetic pathway is the conversion from 5-aminoimidazole ribonucleotide (AIR) to 4-carboxy-5-aminoimidazole ribonucleotide (CAIR). This reaction is