



FIGURE 6: Surface representation of the active site entrance in cystathionine β -synthase. Homocysteine and the putative aminoacrylate intermediate are represented as ball-and-stick models with no surface, while the surface of cystathionine β -synthase is shown with residues lining the active site being labeled and depicted in ball-and-stick representation. Subunit B of the dimer is rendered in ribbons and colored yellow. The loop that holds the PLP in the active site is shown crossing over the pyridinium ring of PLP, which is located at the bottom of the pocket. Homocysteine has been modeled into the rim of the active site analogous to the putative entrance site of indole in tryptophan synthase, which uses a number of nonpolar aromatic amino acids in both its α and β subunits to guide indole toward the PLP-bound aminoacrylate. A number of polar residues line the mouth of the active site of cystathionine β -synthase, and homocysteine could approach from a number of angles. The surface was calculated using GRASP with a 1.4 Å probe radius and 1.5 grids/Å. The surface was input to povscript+ (T. Fenn, <http://people.brandeis.edu/~fenn/povscript/povscript.php3>), where it was oriented with the model, and then rendered with POV-ray.

nm and a broad $\alpha\beta$ absorption band centered at ~ 550 nm (not shown), consistent with the presence of heme in the ferric state. However, the heme content of both mutants was low as indicated by the ratio of the 280 nm (protein) to 428 nm (heme) absorptions, which is 0.92 in the wild-type enzyme and was 0.46 and 0.32 in the C272A and C275S mutants, respectively. The ~ 2 -fold lower heme content of the mutant enzymes correlated with their ~ 2 -fold lower specific activities as compared to the wild-type enzyme (Table 2). In addition, both mutants showed an ~ 2 -fold higher activity in the presence of the allosteric effector, AdoMet, as observed with the wild-type enzyme (not shown).

Importantly, like the wild-type enzyme, which shows a 1.5–2-fold diminution in enzyme activity in the presence of reductants such as dithionite or titanium citrate, the C272A and C275S mutants displayed similar redox sensitivity (Table 2). These results exclude the role of the vicinal cysteines in the redox responsiveness of the human enzyme. Furthermore, these results are consistent with our previous observation that a two-electron reductant, viz., dithiothreitol, which should reduce an accessible disulfide but not the heme, does not affect the activity of the enzyme under anaerobic

Table 2: Relative Activities of Cystathionine β -Synthase Variants in the Presence and Absence of Reductant

enzyme	AdoMet	oxidized ^a	reduced	x-fold change
wild type	+	403 \pm 75	235 \pm 81	1.7
wild type	–	204 \pm 22	146 \pm 22	1.4
C272A	+	226 \pm 15	151 \pm 21	1.5
C275S	+	212 \pm 13	114 \pm 8	1.9
CBS- Δ N69	–	55 \pm 7	50 \pm 9	

^a Enzyme activity was measured in the standard radioactive assay under anaerobic conditions in the presence (reduced) or absence (oxidized) of 1 mM titanium citrate and is given in units of μmol of cystathionine formed min^{-1} (mg of protein) $^{-1}$ at 37 °C.

conditions (6). It should be noted that while the CXXC motif is solvent exposed in the structure of the truncated enzyme, its accessibility in the full-length enzyme is not known and may be significantly different.

Redox Sensitivity of the Δ N69-Hemeless Variant of Human Cystathionine β -Synthase. Residues C52 and H65 serve as the axial ligands to the heme in cystathionine β -synthase (9). Deletion of the N-terminal heme-binding domain in the