



complex provided indispensable guidance for the site-directed mutagenesis studies. A number of residues form the phosphate-binding pocket, including Gly171, Lys172, Gly204, Gly205, Val206, Arg207, Gly236, Ser238, and Ser239 (Figure 11.10). Notably, only the side chain of Ser238 forms a direct hydrogen-binding contact with the phosphate portion of 2-deoxyribose-5-phosphate. The mutant Ser238Asp proved to be the most valuable of site-specific mutants of five different residues that were examined. It was expected that the introduction of a negative charge in very close proximity to the phosphate group would result in electrostatic repulsion and a marked decrease in the affinity of the enzyme for its natural substrate. This was indeed the case. For the reverse (retro-aldol) reaction catalyzed by the Ser238Asp mutant, the K_m for D-2-deoxyribose decreased by about 30% and the k_{cat} doubled. At the same time, the k_{cat} for the phosphorylated substrate dropped 100-fold, and the K_m increased more than 50-fold. Overall, the Ser238Asp mutant showed a 2.5-fold enhancement in catalytic rate toward 2-deoxyribose. Further experiments showed that these data were predictive of the synthetic capabilities of the mutant DERA. The improvements in the aldol reaction activity paralleled the retro-aldol kinetic data.

An unanticipated bonus was that the Ser238Asp mutant enzyme showed an enhanced tolerance for unnatural substrates. It catalyzed a novel sequential aldol reaction using 3-azidopropinaldehyde as the first acceptor and two moles of acetaldehyde as donors to form an azidopyranose, a key intermediate in the synthesis of Lipitor (see Figure 11.5C). 3-Azidopropinaldehyde is not a substrate for the wild-type enzyme.

FIGURE 11.10

Wild-type D-2-deoxyribose-5-phosphate aldolase interactions with D-2-deoxyribose-5-phosphate, as seen in the covalent carbinolamine intermediate in the enzyme-substrate complex at 1.05 Å resolution. Hydrogen bonds are indicated by dotted lines and lengths are given in angstroms. [Heine, A., DeSantis, G., Luz, J. G., Mitchell, M., Wong, C-H., and Wilson, I. A. (2001). Observation of covalent intermediates in an enzyme mechanism at atomic resolution. *Science*, 294, 369–374.]