



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 sub-

**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.]