6.4 Effect of Secondary Mutations on the Stability of TEM-Type β-Lactamases

About 80% of substitutions in TEM-type β-lactamases are secondary, and for most of them the functional role has not been established. The prevalence of these substitutions varies greatly: mutations of some residues (Q39, M182, T265) are common (Figure 6.4), but most of the mutations are rare, occurring in 1–2 enzymes. As a rule, if these mutations are single, they do not affect significantly either the catalytic properties or the substrate specificity of the enzyme. The feature of these mutations is that they are most often combined with the key ESBL mutations (Figure 6.5).

The residue W165 is located at the beginning of the Ω-loop, which forms the entrance to the active site of the enzyme, and it precedes the residue E166, which is involved in coordinating the water molecule during deacylation (Figure 6.3). It is the only one of the four tryptophan residues susceptible to mutations. In TEM-type β-lactamases, the residue W165 is substituted by several amino acids (arginine, cysteine, glycine, and leucine) (Guthrie et al. 2011). The mutant W165R exhibits a slight decrease in the inhibitory effect of clavulanic acid. Molecular modeling suggests that the side chain of R165 is able to form an additional salt bond with the E168 of Ω-loop (Chaïbi et al. 1999).

One of the most studied secondary mutations is the M182T substitution found in 29 TEM-type β-lactamases. It is frequently found in combination with substitutions that have a deleterious effect on thermal stability of the protein (e.g. E104K). The residue M182 is located in the primary sequence at the beginning of the H8 α-helix after the Ω-loop. It has a contact with a β-strand (62–65) leading to residue M69 behind the oxyanion pocket and forms an additional hydrogen bond with A185 (Wang et al. 2002b). It is the first mutation in TEM-type β-lactamases, which was shown to increase the thermodynamic stability of the enzyme by 6.5 °C. Thus, it was called as a global suppressor that compensates for decreasing protein stability caused by the key mutations (Brown et al. 2010; Zimmerman et al. 2017).

Other secondary mutations located far from the active site were actively investigated in relation to their effects on the thermal stability of the protein globule. For T265M mutation, similar properties were first predicted (Salverda et al. 2010). Analysis of its location in the protein globule showed that the hydroxyl group of threonine forms a hydrogen bond with R43. When replacing with M265, the hydrogen bond disappears, and the distance between S70 and M265 increases. The long side chain of methionine fills the hydrophobic region inside the protein globule, located next to it. The hydrophobic interaction of aliphatic residues leads to compaction of the globule, which lowers the free energy of the protein and compensates for the disappearance of the hydrogen bond. Compaction of the protein globule may lead to an increase in the