of irregular structure (SDN loop [S130, N132], Ω-loop [G166, N170]) (Figures 6.1 and 6.2).

Figure 6.3 shows the scheme of enzymatic hydrolysis of β-lactam antibiotics, which includes three main processes: binding (i), acylation (ii), and deacylation (iii). These stages can be described by a set of kinetic parameters (K_M and k_{cat}), which are a function of the kinetic constants of the elementary stages of binding (k_1, k_{-1}), acylation (k_2), and deacylation (k_3) (Raquet et al. 1994). At the first stage, the binding of an antibiotic molecule occurs in the active site of the enzyme. The main process is the deprotonation of the catalytic S70 with the participation of the Ω-loop residues E166 and N170, which form a network of hydrogen bonds with the water molecule (Stec et al. 2005). The alternative proton transfer pathway includes the participation of residues K73 and S130 (Massova and Kollman 2002). As a result of the nucleophilic attack of deprotonated S70 on the carbonyl group of the antibiotic, a high-energy acyl intermediate is formed. The charged side groups of residues K234 and N132, which form ionic bonds with the charged groups of the antibiotic, are involved in its stabilization, as well as residue A237, which stabilizes the negative charge on the carbonyl carbon atom.

In the second stage, the transition of the intermediate to the low-energy covalent acyl-enzyme complex occurs, wherein the nitrogen atom is protonated and the amide bond of the β-lactam ring of the antibiotic is cleaved.

In the process of deacylation (stage 3), the water molecule coordinated by El66 and N170 attacks the carbonyl bound to the serine oxygen, and this bond is hydrolyzed. As a result, an enzyme molecule and a hydrolyzed antibiotic molecule are released.

Amino acid residues S70, K73, K234, E166, and N170 involved in the catalytic cycle of antibiotic hydrolysis are conservative and do not mutate, and residues S130 and A237 are rarely mutating.

6.2.2 Mutations Causing Phenotypes of TEM-Type β-Lactamases

Based on differences in substrate specificity due to amino acid mutations, TEM-type β-lactamases are divided into four phenotypes: 2b, which are β-lactamases hydrolyzing penicillins and first-generation cephalosporins; 2be (ESBL), which are β-lactamases hydrolyzing penicillins, first- to fourth-generation cephalosporins, and monobactams; 2br (inhibitor-resistant type [IRT]), which are β-lactamases resistant to inhibitors of β-lactam structure (clavulanic acid, sulbactam, tazobactam); and 2ber (complex mutation type [CMT]), which are inhibitor-resistant ESBLs, mixed type (Bush and Jacoby 2010). The mutations are divided into several types according to their influence on the catalytic properties of β-lactamases: the key mutations, which change the phenotype, and secondary mutations, which affect mainly the stability and folding of the protein globule. Secondary mutations do not change the phenotype; some of them exhibit a compensatory suppressor role (Zimmerman et al. 2017).