Indian Journal of Biochemistry & Biophysics
Vol. 60, September 2023, pp. 750-755
DOI: 10.56042/ijbb.v60i9.4029

The role of water molecules and its dynamics to the binding site of β-lactamase enzyme with respect to β-lactamase inhibitor

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Received 11 March 2023; *revised 15 July 2023*

β-lactamase hydrolyses amide bond of β-lactam ring. β-lactam antibiotics are the main arsenal in antibiotic regime. High degree of mutation and genetic variation in β-lactamase enzymes have elevated the resistance towards β-lactamase inhibitors. There are 4 classes of β-lactamase, namely Class A, C, and D which are Serine proteases and Class B is the Metallo proteases. The most documented one is TEM**,** where 4 water molecules (315, 319, 440, 441) play an important role in the binding site. In the present work we have tried to explain the involvement of water molecule with respect to docking behavior with S70 (Serine), E166 (Glutamate) and N170 (Asparagine) along with an important Ω – loop (R161-D179) which allosterically modulate the behavior of the binding site. This will aid us to identify potential candidates as novel antibiotics precisely interacting with the substrate binding site and Ω – loop of β-lactamase and their interaction with these 4 water molecules. We have docked Clavulanic acid (CA), Sulbactam (SB), Tazobactam (TB) with wild (1ZG4) and mutated (1ZG6) TEM in the presence and absence of HOH, which justifies the importance of water molecules playing an important role in the hydrolysis of β-lactam as well as modulating the binding affinity of a potential drug candidate.

Keywords: Antimicrobial resistance (AMR), Docking, Water mediated interactions, β-lactamase inhibitors, β-lactamase

β-lactamases are serine proteases family enzymes of the hydrolytic class A group that are produced by the various bacteria. This enzyme hydrolyzes the amide bond of the β-lactam ring which is found in the β-lactam antibiotics. Over a period of time, bacteria continue to evolve by acquiring new genes as well as mutations in the existing genes. This leads to an increase in the resistance of every class of β-lactam antibiotics posing a serious threat to human health[1].

To combat this resistance, β-lactam antibiotics are co-administered with β-lactam inhibitors such as amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, ceftazidime/avibactam, imipenem/relebactam, meropenem/vaborbactam[2]. These inhibitors work through two main mechanisms based on their mode of action. Clavulanic acids sulbactam and tazobactam are the competitive inhibitors, which permanently inactivate the enzyme via binding to the substrate binding site. Whereas, avibactam and relebactam are non-competitive inhibitors, which destabilize the enzymes *via* forming a sterically unfavorable interaction[3].

Overall, these inhibitors improved the treatment of β-lactam antibiotics but a prolonged use of β- lactamase inhibitors with antibiotics has developed resistance to inhibitor molecules as well. Hence, there is an urgent need for developing novel inhibitors for the effective treatment against infection by resistant bacteria[4].

For designing a novel inhibitor molecule, we need to understand the mechanism of the hydrolysis of the inhibitor molecule by β-lactamase. In this hydrolysis of the inhibitor molecule by β-lactamase, apart from some important amino acids at the binding site - S70 (Serine), E166 (Glutamate) and N170 (Asparagine), and Ω – loop (R161-D179), water molecules are also playing a crucial role by deprotonating S70[5]. All the important amino acids are depicted in the (Fig. 1).

Studies done so far state that the importance of water molecules has been discussed with various proteins and enzymes, but there are still gaps where researchers are not considering the water molecules during docking studies[6-9]. In order to understand the role of water molecules in this hydrolysis, we selected a wild-type (1ZG4) TEM-1, which is sensitive to β- lactamase inhibitors, and mutant (1ZG6) TEM-1,

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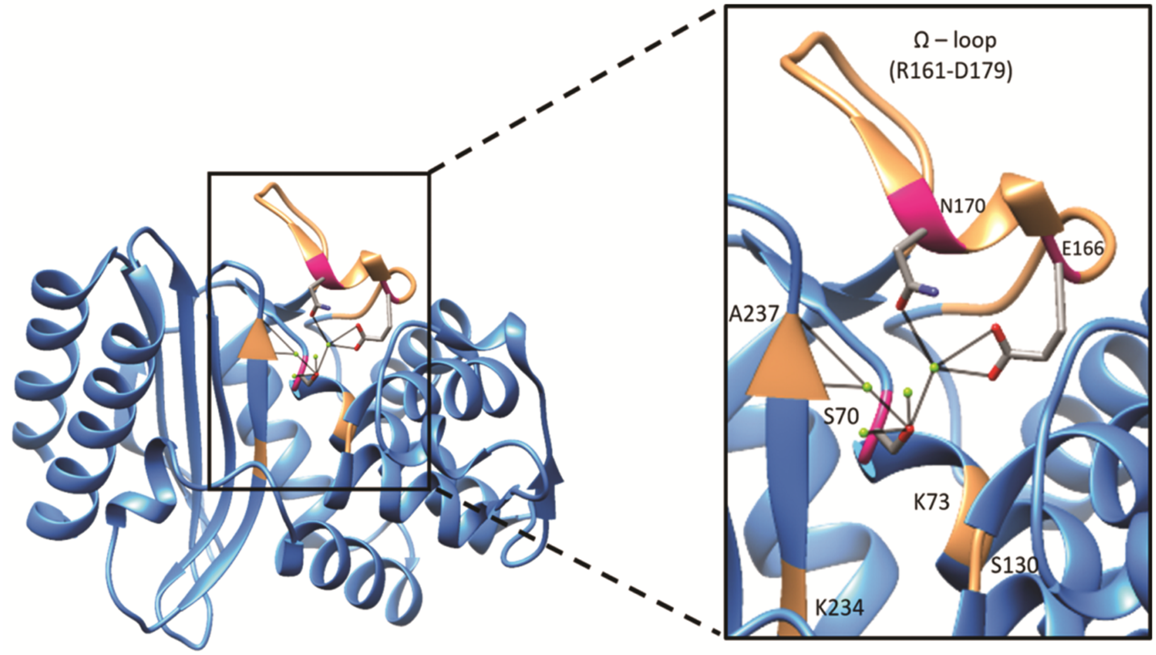


Fig. 1 — Substrate Binding Sites (Deep Pink Ribbon) and Omega Loop (Sandy Brown) With HOH (Chartreuse) of TEM-1 B-Lactamase (PDB Id: 1ZG4 – Wild Type-Dodger Blue)

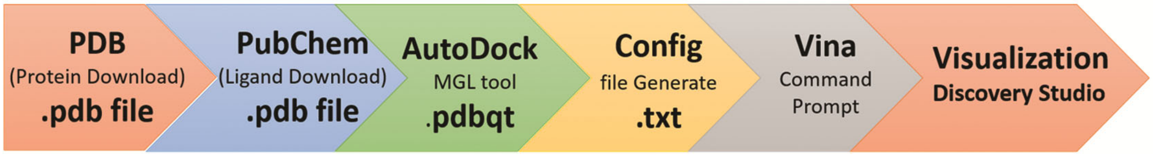


Fig. 2 — Overview of the methods used in docking studies

which is resistant to β-lactamase inhibitors (Fig. 1). In mutant (1ZG6) TEM-1, S70 (Serine) is replaced by the G70 (Glycine), Other than that all of the sequences are similar (Fig. 3). In the present work, we carried out a docking study of β-lactam inhibitors with 1ZG4 and 1ZG6 to see the involvement of water molecules at the active site.

The Sequential difference between 1ZG4 and 1ZG6, important residues for binding – Figure 1 shows all the inhibitors are having a same binding site.

Material and Methods

Hardware

The entire study was executed on HP Laptop with an Intel(R) Core(TM) i5-5200U CPU 2.20 GHz processor, 4 GB RAM, 240GB SSD, and a 64-bit operating system. All software utilized for the present study were open-source tools and freely downloadable. The flowchart of the method is shown in (Fig. 2).

Structure and Sequence retrieval

The protein structures and sequences of wild-type TEM-1 (PDB ID: 1ZG4) and Mutant TEM-1 (PDB ID: 1ZG6) were downloaded from RCSB-PDB (Research Collaboratory for Structural Bioinformatics-Protein Data Bank) database in a .pdbformat[10]. The ligand structures Clavulanic acid, Sulbactam, and Tazobactam were downloaded from PubChem database in a 3D Conformer (.SDF Format)[11].

Multiple Sequence Alignment (MSA)

MSA of wild type (PDB ID: 1ZG4) and Mutant (PDB ID: 1ZG6) was carried out using Jalviewv2.11.1.0 using default parameter[12]. Further, Chimera was used to predict the overall RMSD value between wild-type (1ZG4) TEM-1 and mutant (1ZG6) TEM-1. RMSD mainly explains the differences between two protein structures[13].

Docking studieS

We prepared the ligand and protein (with and without water molecules) using MGL tool in

Autodock Vina 4.2.6. Using the tool we have added hydrogen atoms, Kollman charges, and using default solvation parameters we have generated a .pdbqt file. The computational molecular docking of Wild type TEM-1 (PDB ID: 1ZG4) and Mutant TEM-1 (PDB ID: 1ZG6) with the ligand (Clavulanic acid (CA), Sulbactam (SB), Tazobactam (TB)) was performed in the presence and absence of water molecule using AutoDockVina[14-16]. In all the docking processes, we have used blind docking covering the whole protein in the grid box. The best docking model was selected based on the lowest energy. The lowest energy docked model implied the highest binding affinity because of the highest stability of conformation.

Visualization studies

The 2D interactions of the docked complexes were analysed using Discovery studio v20.1.0.19295[17] whereas 3D models were analysed using UCSF Chimera v1.11.2, respectively[13,18].

Results and Discussion

We performed multiple sequence alignments of 1ZG4 and 1ZG6 using Jalview and found only one important amino acid substitution in substrate binding site (Serine to Glycine at 70) (Fig. 3A). This helps us to understand the relationship of water molecules with Serine 70 in the hydrolysis mechanisms of β- lactamase. The overall RMSD value of superimposed structure of wild-type (1ZG4) TEM-1 and mutant (1ZG6) TEM-1 was found to be 0.218 Å (Fig. 3B).

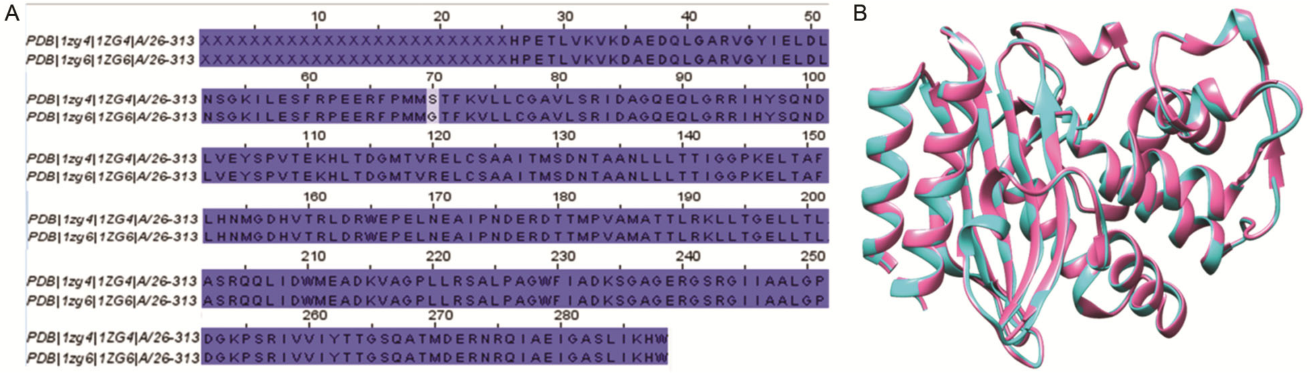
To understand the role of water molecules we carried out 12 dockings using three β-lactamase inhibitors (CA, SB, and TB) as ligands with two β-lactamases - 1ZG4 and 1ZG6, both in the presence and absence of water molecules. We found that in absence of water molecules, both in wild type as well as in mutants, CA, SB, and TB are interacting with more number of amino acids (E166, K73, S130, K234, A237, S70, N170, and Omega loop R161- D179) while the presence of water molecules decreases the interaction between inhibitor ligand and 1ZG4 / 1ZG6 (Fig. 4).

In presence of water molecules, 1ZG4 has no interaction with water molecules while 1ZG6 has an interaction with four water molecules (HOH 315, HOH 319, HOH 440, and HOH 441) which are present in the substrate binding site. Figure 4 also shows the decrease in interaction of ligands with important amino acids in presence of water molecules. The interactions and bond details with ligands and receptors are described in the figures.

Docking results of 1ZG4 and 1ZG6 with CA, SB,and TB indicate interaction with the β-lactam ring in absence of water, whereas in presence of water molecules wild type 1ZG4 has an interaction with only β-lactam ring, while 1ZG6 mutant have an interaction with β-lactam ring and water molecules as well.

Without water molecules, CA forms six conventional hydrogen bonds with reported amino acid and one Pi-sigma bond, while in presence of water, it retains three conventional hydrogen bonds (N132, S130, K234) and one Pi-sigma bond (Y105) (Fig. 4 i-a and i-b). The pattern is similar into sulbactam (SB) (Fig. 4 ii-a and ii-b) as well as tazobactam (TB) (Fig. 4 iii-a and iii-b).

Without water molecules, CA forms three conventional hydrogen bonds with reported amino acid and three carbon-hydrogen bonds (N170, K234, K73), while in presence of water, it increases conventional hydrogen bonds to four (N170, N132, S130, K73) and three water hydrogen bonds (HOH315, HOH440, HOH441) in (Fig. 4 i-c and i-d). The pattern is found similar for sulbactam (SB) (Fig. 4 ii-c and ii-d) as well as tazobactam (TB) (Fig. 4 iii-c and iii-d).



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                                 10        20        30        40        50
PDB|1zg4|1ZG4|A/26-313 XXXXXXXXXXXXXXXXXXXXXXXXXHPETLVKVKDAEDQLGARVGYIELDL
PDB|1zg6|1ZG6|A/26-313 XXXXXXXXXXXXXXXXXXXXXXXXXHPETLVKVKDAEDQLGARVGYIELDL
                                 60        70        80        90       100
PDB|1zg4|1ZG4|A/26-313 NSGKILESFRPEERFPMMSTFKVLLCGAVLSRIDAGQEQLGRRIHYSQND
PDB|1zg6|1ZG6|A/26-313 NSGKILESFRPEERFPMMGTFKVLLCGAVLSRIDAGQEQLGRRIHYSQND
                                110       120       130       140       150
PDB|1zg4|1ZG4|A/26-313 LVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAF
PDB|1zg6|1ZG6|A/26-313 LVEYSPVTEKHLTDGMTVRELCSAAITMSDNTAANLLLTTIGGPKELTAF
                                160       170       180       190       200
PDB|1zg4|1ZG4|A/26-313 LHNMGDHVTRLDRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTL
PDB|1zg6|1ZG6|A/26-313 LHNMGDHVTRLDRWEPELNEAIPNDERDTTMPVAMATTLRKLLTGELLTL
                                210       220       230       240       250
PDB|1zg4|1ZG4|A/26-313 ASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGP
PDB|1zg6|1ZG6|A/26-313 ASRQQLIDWMEADKVAGPLLRSALPAGWFIADKSGAGERGSRGIIAALGP
                                260       270       280
PDB|1zg4|1ZG4|A/26-313 DGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
PDB|1zg6|1ZG6|A/26-313 DGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW
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Fig. 3 — (A) Multiple sequence alignment of 1ZG4 (Wild Type) and 1ZG6 (Mutant); and (B) Superimpose structure of 1ZG4 (Wild Type) highlighted in Cyan and 1ZG6 (Mutant) Highlighted in Hot Pink

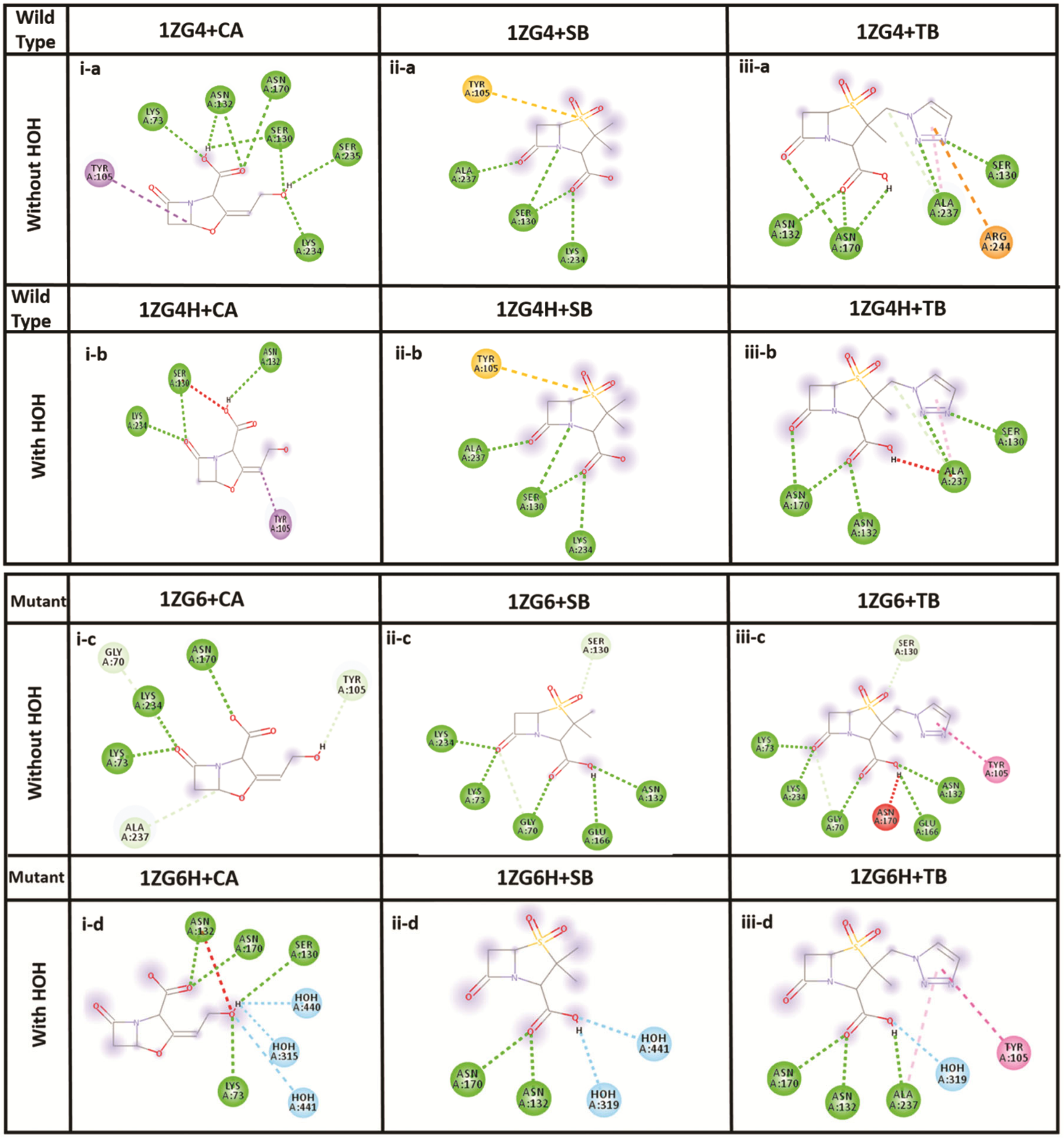

Fig. 4 — Docked Complexes Of Clavulanic Acid (Ca), Sulbactam (Sb), and Tazobactam (Tb) With The 1ZG4/1ZG6 In Absence of HOH (Figure I - A, Ii –A, Iii –A/ Figure I-C, Ii-C, Iii-C) & In Presence of HOH (1ZG4H/1ZG6H) (Figure I - B, Ii –B, Iii –B/Figure I - D, Ii –D, Iii –D) Docked Complexes Comprising Of Conventional Hydrogen Bonds (Green), Pi-Sigma (Purple), Pi Cation (Orange), Pi-Pi T-Shaped (Dark Pink), Water Hydrogen Bond (Light Blue),Carbon–Hydrogen Bonds(Light Green), and Unfavourable Donor-Donor (Red) Interactions

In our docking study, when we removed the water molecules, we observe that all the inhibitors were interacting with wild type as well as mutant. But physically this is not accurate and gives us wrong information[19]. While, if we consider the water molecules in the docking study, we observe that water molecules are interacting with mutants, however, this may not lead to the hydrolysis of β-lactam inhibitors (Table 1). Interaction with water molecules in docking doesn't mean it hydrolyses the ligand. As reported earlier, water molecules help to deprotonate S70 and free it to interact with β-lactam rings which help in hydrolysis of β-lactam[19,20].

Table 1 — Summary of the docking study in presence and absence of water molecule with respect to hydrolysis of β-lactam ring

	1ZG4 (WT)	1ZG6 (Mutant)	
With HOH	β-lactam ring	✓	✗
	HOH	✗	✓
Hydrolysis of β-lactam ring	✓	✗	
	1ZG4 (WT)	1ZG6 (Mutant)	
Without HOH	β-lactam ring	✓	✓
Hydrolysis of β-lactam ring	✓	✓	

Conclusion

Water molecules play a pivotal role in reactions like condensation and hydrolysis, as water takes part in the transition state (intermediated), where it helps in stabilization of the protein through the hydrogen bond. It also contributes to enthalpy and entropy stabilization[21]. During the docking process, providing a cell-like environment will help us to find the best lead molecule using a computational tool.

These results are also supporting the reported documents, where if we consider the water molecule while docking the ligand, we can get rid of the false positive results and increase the accuracy of the results[22,23]. Robert *et al*. (2008) described ligand-protein docking with and without the presence of water molecules. They discovered that incorporating water molecules in the binding site improves precision in docking and simulation studies significantly[24].

Acknowledgement

The authors would like to express their sincere gratitude to the Department of Biochemistry, The Maharaja Sayajirao University of Baroda for arranging the Two Days International Conference on "Celebrating Proteins on the Birth Centenary of Dr. G. N. Ramachandran" (CPBCR) on 3rd and 4th March 2023 and giving us the platform to present our work in the conference. We are also grateful to Gujarat State Biotechnology Mission (GSBTM) and Department of Biotechnology (DBT), India AMR (GSBTM JD/(R&D) 618/21-22) for giving a chance to work on state and national-level surveillance program and providing financial support for the same. We are also thankful to Gujarat State Fertilizer and Chemical University Vadodara, Gujarat, India for providing the platform to conduct the present study.

Conflict of interest

All authors declare no conflict of interest.

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