Stabilizing Role of Water Solvation on Anion–π Interactions in Proteins

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ABSTRACT: In this work, anion–π interactions between sulfate groups (SO₄²⁻) and protein aromatic amino acids (AAs) (histidine protonated (HisP), histidine neutral (HisN), tyrosine (Tyr), tryptophan (Trp), and phenylalanine (Phe)) in an aqueous environment have been analyzed using quantum chemical (QC) calculations and molecular dynamics (MD) simulations. Sulfates can occur naturally in solution and can be contained in biomolecules playing relevant roles in their biological function. In particular, the presence of sulfate groups in glycosaminoglycans such as heparin and heparan sulfate has been shown to be relevant for protein and cellular communication and, consequently, for tissue regeneration. Therefore, anion–π interactions between sulfate groups and aromatic residues represent a relevant aspect to investigate. QC results show that such an anion–π mode of interaction between SO₄²⁻ and aromatic AAs is only possible in the presence of water molecules, in the absence of any other cooperative non-covalent interactions. Protonated histidine stands out in terms of its enhancement in the magnitude of interaction strength on solvation. Other AAs such as non-protonated histidine, tyrosine, and phenylalanine can stabilize anion–π interactions on solvation, albeit with weak interaction energy. Tryptophan does not exhibit any anion–π mode of interaction with SO₄²⁻. The order of magnitude of the interaction of aromatic AAs with SO₄²⁻ on microsolvation is HisP > HisN > Tyr > Trp > Phe. Atoms in molecules (AIM) analysis illustrates the significance of water molecules in stabilizing the divalent SO₄²⁻ anion over the π surface of the aromatic AAs. MD simulation analysis shows that the order of magnitude of the interaction of SO₄²⁻ with aromatic AAs in macroscopic solvation is HisP > HisN, Tyr, Trp > Phe, which is very much in line with the QC results. Spatial distribution function analysis illustrates that protonated histidine alone is capable of establishing the anion–π interaction with SO₄²⁻ in the solution phase. This study sheds light on the understanding of anion–π interactions between SO₄²⁻ and aromatic AAs such as His and Tyr observed in protein crystal structures and the significance of water molecules in stabilizing such interactions, which is not feasible otherwise.

1. INTRODUCTION

Non-covalent interactions play an important role in stabilizing the structural organization of life, ranging from small molecules in chemistry to huge assemblies in biological systems and crystals. Furthermore, water-mediated solvation and ions also play an important role in the function and regulation of biological systems. Thus, understanding the intricate details of non-covalent interactions and the role of solvation remains an important research area in the field of natural sciences. Among such non-covalent interactions, electrostatics play a quite relevant role, particularly in hydrogen bonding, which is well characterized. Cation–π and π–π interactions are well characterized, whereas anion–π interactions are not as well characterized as the other classical non-covalent forces.

Anion–π interactions are a relatively new type of non-covalent forces observed between an anion and an electron-deficient aromatic (π) system. These interactions are relatively weak when compared to other non-covalent forces including H-bonding and cation–π interactions; however, they are present in nature and have therefore also a certain importance in biology and chemistry, particularly in the fields of molecular engineering and materials science. A survey on anion–π interactions in the structures available in the Protein Data Bank (PDB) has shown that these interactions exist as a reasonable common motif. Structural analysis of such anion–π interactions in proteins showed that they are mostly present in buried regions and that they clearly have a cooperativity effect with other non-covalent interactions such as cation–π or π–π. Anion–π interactions have been reported to be responsible for the stabilization of the small WW protein domain [WW domains contain ~40 amino acids (AAs) with two tryptophan residues (labeled W in the single-letter AA code, hence the name WW domain)] that bind to...
short proline-rich sequences containing PPXY, PPLP, or PPR motifs and the Sm/LSm protein family [Sm and Sm-like (LSm) proteins form complexes involved in various RNA-processing events].18,19 Furthermore, anion–π interactions have been recently exploited in protein-based catalyst design.20 Nanomolar detection of sulfide ions using halo-substituted subphthalocyanines exploiting the anion–π interaction was recently demonstrated.21

One significant important aspect observed from previous studies by Lucas and co-workers is the anion–π interaction between sulfates (SO4^{2−}) and aromatic AAs histidine (His) and tyrosine (Tyr) without any further non-covalent interactions stabilizing them.17 Previous studies have shown the relevance of sulfate groups in glycosaminoglycans (GAG), such as heparin and heparan sulfate, for their interaction with proteins of the extracellular matrix such as cytokines and growth factors and therefore important regulators of cellular communication and tissue regeneration.22–25

The relevant role of solvent medium in the recognition of sulfated GAG by proteins has been also reported.26–29 These studies have mainly focused on the interaction of sulfate groups with positively charged AAs (i.e., Lys and Arg). The importance of anion–π interactions and the role of solvation are not characterized well yet in the sulfate–aromatic systems; particularly, the suitability of the current force fields in describing the anion–π interactions needs to be investigated. This will bring forth the importance of anion–π interactions in protein–anionic molecular systems. In the recent past, we have also exemplified the significance of solvation in the cation–π interaction between the guanidinium cation and aromatic AAs to understand protein denaturation by guanidiniumchloride.30

Here, we address several scientific questions. First of all, how a highly negatively charged divalent anion, SO4^{2−}, stabilizes over aromatic π rings and how this may happen without any additional cooperative stabilizing component. Second, what stabilizes the SO4^{2−} over a proteogenic aromatic AA, as generally anion–π interactions are observed in chemical systems and crystals between an anion and an electron-deficient π system such as halogenated benzene. However, such a scenario does not exist in the aromatic side chains of the protein AAs. Third, in contrast to buried anion–π interactions mostly observed in proteins, sulfate ions used as solvents in protein purification and crystallization processes or sulfate groups in GAG may interact with solvent-exposed aromatic residues. In such cases, how the molecular interactions are stabilized. Fourth, we inspect why these kinds of SO4^{2−}–π interactions are observed only in His and Tyr and not in other aromatic AAs such as phenylalanine (Phe) and tryptophan (Trp).17

We explore the role of water molecules in stabilizing the anion–π interaction between SO4^{2−} and aromatic AAs such as His (HisP and HisN for protonated and non-protonated, respectively), Phe, Tyr, and Trp using quantum chemical (QC) calculations and molecular dynamics (MD) simulations with the ultimate goal of shedding light on how the sulfate ions are stabilized in His and Tyr residues in proteins and not with other aromatic AAs. The nanomechanics of anion–π interactions in aqueous medium have also been experimentally validated recently.31

### 2. RESULTS AND DISCUSSION

#### 2.1. Quantum Chemistry Results

The interaction energies of the aromatic AAs with the different hydration patterns of SO4^{2−}–Wn (n = 1–12) obtained from QC calculations are presented in Table 1, and selected geometries are illustrated in Figure 1. Analysis of these geometries indicates that in all the cases, SO4^{2−} interacts with the AAs on their aromatic ring circumference with a lesser number of

| AA   | LE   | W1   | W2   | W3   | W4   | W5   | W6   |
|------|------|------|------|------|------|------|------|
| HisP | 23.02| 23.85| 21.52| Y    | 199.13| 190.77| 185.93| Y    |
| HisN | 28.67| 22.55| 39.44| Y    | 34.32 | 33.01 | 29.33 | Y    |
| Tyr  | 45.44| 39.18| 35.52| Y    | 33.55 | 28.21 | 23.86 | Y    |
| Phe  | 20.80| 18.49| 13.33| Y    | 13.05 | 9.07  | 8.59  | Y    |
| Trp  | 40.79| 36.53| 33.80| Y    | 31.25 | 27.65 | 25.34 | Y    |

| AA   | LE   | W7   | W8   | W9   | W10  | W11  | W12  |
|------|------|------|------|------|------|------|------|
| HisP | 185.76| Y    | 186.04| Y    | 172.49| Y    | 172.66| Y    |
| HisN | 28.63| 26.66| 27.73| Y    | 27.66| 27.72| 27.49| Y    |
| Tyr  | 26.88| 20.70| 21.62| Y    | 20.94| 20.03| 19.57| Y    |
| Phe  | 7.16 | 8.09 | 6.89 | Y    | 6.67 | 6.67 | 6.82 | Y    |
| Trp  | 24.04| 22.93| 21.97| Y    | 20.80| 19.98| 18.97| Y    |

Note: The presence of the anion–π mode of interaction between SO4^{2−} and π of aromatic AAs based on distance and angle criteria is marked as Y next to the LE Values. (The values presented in bold represent SO4^{2−} conversion to HSO4^{−} during optimization.)
water molecules and that it moves toward the anion–π arrangement on increasing solvation except in the case of tryptophan. In Table 1, we also categorize the presence of anion–π interactions based on the distance and angle criteria.
between the SO₄²⁻ and plane of the aromatic ring of the AA based on a previous definition (d = 2–5 Å and α ≥ 50°)\textsuperscript{15}. Conversion of SO₄²⁻ into HSO₄⁻ during the optimization process is highlighted in Table 1, and it is observed that such conversion occurs only in the case of HisP and HisN with Wₙ (n = 1–2). Multiple protonation states of histidine based on Table 2. Interaction Energy Values (in kcal/mol) of Selected Systems with M06-2x/6-31+g* and with GD3 Dispersion Corrections

|   | LE     | LE (GD3) | LE     | LE (GD3) | LE     | LE (GD3) | LE     | LE (GD3) |
|---|--------|----------|--------|----------|--------|----------|--------|----------|
| HisP | −23.02 | −23.44   | −199.13| −199.97  | −186.04| −187.40  | −162.66| −164.17  |
| HisN | 28.67  | 28.30    | −34.32 | −35.03   | −26.66 | −27.88   | −27.49 | −28.88   |
| Tyr  | −45.44 | −45.89   | −33.55 | −34.11   | −20.70 | −22.13   | −19.57 | −21.08   |
| Phe  | −20.80 | −21.30   | −13.05 | −13.75   | −8.09  | −9.47    | −6.82  | −8.31    |
| Trp  | −40.79 | −41.28   | −31.25 | −31.84   | −22.93 | −23.66   | −18.97 | −19.72   |

Table 3. Values of the Distance (in Å) and Angle (in deg) for Each Anion–π Studied System

|   | W3     | W4     | W5     | W6     | W7     | W8     | W9     | W10    | W11    | W12    |
|---|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| HisP | 3.85/54 | 2.69/91 | 2.82/88 | 3.07/81 | 2.80/83 | 2.74/83 | 2.83/84 | 3.08/76 | 2.82/81 | 2.87/84 |
| HisN | 3.86/50 | 3.02/71 | 3.02/72 | 3.03/72 | 3.05/72 | 3.95/125| 3.98/125| 4.02/124| 4.09/124| 4.09/124|
| Tyr  | 3.03/92 | 3.01/90 | 3.10/82 | 3.36/74 | 3.41/78 | 3.56/78 | 3.63/78 | 3.59/77 |        |        |
| Phe  |        |        |        |        |        |        |        |        |        |        |
| Trp  |        |        |        |        |        |        |        |        |        |        |

Figure 3. Molecular graphs of aromatic AAs–SO₄²⁻–Wₙ (n = 1, 4, 8, and 12) clusters.
Table 4. Values of Electron Density ($\rho(r_c)$) and Its Laplacian ($\nabla^2\rho(r_c)$) at the BCPs of SO$_4^{2-}$ and W Molecules with the aromatic AAs in Selected Systems$^a$

| AA  | SO$_4^{2-}$ $\rho(r_c)$ | W | SO$_4^{2-}$ $\nabla^2\rho(r_c)$ | W |
|-----|-------------------------|---|-------------------------------|---|
| W1  | 0.0400, 0.0089/0.489    | 0.0090 | -0.0309, -0.0085/0.0394 | -0.0077 |
| W4  | 0.0140, 0.0129, 0.0129/0.0398 | 0.0595, 0.0123, 0.0438, 0.0079/0.1235 | -0.0128, -0.0124, -0.0119/0.0371 | -0.0058, -0.0082, -0.0064/0.0352, -0.0129, -0.0114/0.0069 |
| W8  | 0.0147 | 0.0071, 0.0089, 0.0063, 0.0547, 0.0162, 0.0165, 0.0119/0.1207 | -0.0138 | -0.0093, -0.0083, -0.0064, -0.0062, -0.0192, -0.0138, -0.0012, -0.0107, -0.0167/0.0898 |
| W12 | 0.0127 | 0.0102, 0.0079, 0.0045, 0.0073, 0.0247, 0.0135, 0.0016, 0.0105, 0.0158/0.1104 | -0.0124 | -0.0093, -0.0083, -0.0064, -0.0062, -0.0192, -0.0138, -0.0012, -0.0107, -0.0167/0.0898 |

| W1  | 0.0510, 0.0051/0.5417 | 0.0175 | -0.0253, -0.0031/0.0284 | -0.0119 |
| W4  | 0.0047, 0.0022/0.0129 | 0.0081 | -0.0344, -0.0079/0.0423 | -0.0070 |
| W8  | 0.0062 | 0.0370, 0.0131, 0.0073, 0.0210, 0.0063/0.0847 | -0.0052 | -0.0279, -0.0110, -0.0070, -0.0152, -0.0055/0.0666 |
| W12 | 0.0104 | 0.0139, 0.0114, 0.0102, 0.0100, 0.0070, 0.0080, 0.0204, 0.0126/0.0935 | -0.0089 | -0.0132, -0.0120, -0.0104, -0.0076, -0.0065, -0.0070, -0.0144, -0.0094/0.0805 |

| W1  | 0.0198, 0.0191/0.0389 | 0.0118 | -0.0253, -0.0127/0.0272 | -0.0092 |
| W4  | 0.0112 | 0.0173, 0.0068, 0.0114, 0.0057/0.0412 | -0.0086 | -0.0125, -0.0059, -0.0088, -0.0063/0.0335 |
| W8  | 0.0080 | 0.0070, 0.0072, 0.0085, 0.0036, 0.0085, 0.0098/0.0446 | -0.0067 | -0.0067, -0.0076, -0.0079, -0.0031, -0.0083, -0.0088/0.0424 |
| W12 | 0.0059 | 0.0104, 0.0098, 0.0087, 0.0100/0.0389 | -0.0047 | -0.0108, -0.0091, -0.0076, -0.0090/0.0357 |

| W1  | 0.0055, 0.0019, 0.0096/0.0270 | 0.0907, 0.0094/0.1001 | -0.0055, -0.0096, -0.0081/0.0232 | -0.0272, -0.0083/0.0355 |
| W4  | 0.0052, 0.0084/0.0937 | 0.0085, 0.0084, 0.0085, 0.0072/0.0702 | -0.0074 | -0.0073, -0.0098, -0.0302, -0.0061, -0.0073/0.0607 |
| W8  | 0.0082 | 0.0070, 0.0094, 0.0052, 0.0088, 0.0377, 0.0089/0.0770 | -0.0049 | -0.0059, -0.0088, -0.0055, -0.0091, -0.0302, -0.0078/0.0673 |
| W12 | 0.0059 | 0.0052 | 0.0433, 0.0159/0.0592 | -0.0151 | -0.0352 |

| W1  | 0.0403, 0.0130/0.0533 | 0.0343, 0.0119/0.0462 | -0.0352, -0.0013/0.0447 |
| W4  | 0.0403, 0.0130/0.0533 | 0.0343, 0.0119/0.0462 | -0.0352, -0.0013/0.0447 |

pH and their biological role in various processes such as enzyme catalysis are well established$^{32}$ From our results, it is observed that at least three water molecules are required to stabilize the SO$_4^{2-}$ in its divalent anionic state in the presence of histidine. The role of water solvation in stabilizing the SO$_4^{2-}$ has been already reported.$^{33,34}$ The obtained LE values for the investigated systems are shown in Figure 2A (for all the AAs) and Figure 2B (for all AAs except HisP).

The analysis of the LE results shows that each aromatic AA behaves differently with the SO$_4^{2-}$–W$_n$ clusters in terms of their LE magnitude. In general, Phe, Tyr, and Trp act in a similar fashion (i.e., with the LE decrease with the increase in the number of water molecules). The imidazole-containing AA histidine acts differently based on its different protonation states. In the case of HisN, the LE is highly unfavorable for up to two water molecules; it is most favorable at $n = 3$, and then, it decreases gradually when adding further water molecules. In the case of HisP, the results are completely outstanding. Here, there is a decrease in the LE up to three water molecules, and on the addition of the fourth water molecule, there is a huge gain in the LE (i.e., $\sim$199.13 kcal/mol) and then a decrease of up to $\sim$162.66 kcal/mol for the W$_{12}$ cluster. Thus, the obtained LE pattern and its magnitude are remarkable in the case of the protonated histidine when compared to the other aromatic AA systems. It is noteworthy to mention that it is expected that positively charged histidine possesses a better LE with SO$_4^{2-}$ when compared to the other uncharged aromatic AAs, but what is remarkable is that solvation plays such an important role in stabilizing the HisP–SO$_4^{2-}$ to such a high LE value, which is close to the covalent bond limit. To understand the effect of dispersion contribution on the interaction process, we also calculated the LE using dispersion-corrected density functional theory (DFT) for the selected systems. The obtained results (Table 2) show that the inclusion of dispersion contribution changes neither the interaction pattern nor the magnitude of the interaction energy significantly (deviation up to a maximum of $\sim$1.5 kcal/mol), which does not affect any of the observations derived so far.

The distance and angle values of the observed anion–π arrangement of the AA–SO$_4^{2-}$–W$_n$ clusters are provided in Table 3. The analysis of the results obtained from the geometrical analysis along with the energetics (Table 1) shows that HisP requires a minimum of three water molecules to attain the anion–π arrangement and retain it up to 12 water molecules. Following that, Phe requires at least five water molecules to have the anion–π arrangement, albeit their LE is
Obtained from Triplicate of MD Simulation with the de anion SO₄⁻ by Lucas and co-workers demonstrated the presence of protein crystal structures in the Protein Data Bank performed − that the analysis of anion and the SO₄⁻ to 4.09 Å, and the angle between the plane of the aromatic ring analyzed aromatic AAs. Which were found to have some considerable I.E among the water molecules to stabilize the anion very low. HisN and Tyr are shown to require at least eight water molecules to stabilize the SO₄⁻ and aromatic AAs with the increase in −π interactions even when fully saturated with 12 water molecules. The distance between the centroid of the aromatic ring and the SO₄⁻ ranges from 50 to 124°, which is well in line with the definition of an anion−π interaction. The obtained results clearly show that solvation plays an important role in stabilizing the SO₄⁻ over aromatic AAs in the anion−π configuration. It is also noteworthy to mention that the analysis of anion−π interactions in the available protein crystal structures in the Protein Data Bank performed by Lucas and co-workers demonstrated the presence of SO₄⁻−AA anion−π interactions in the case of His and Tyr, which were found to have some considerable I.E among the analyzed aromatic AAs.

### Table 5. Interaction Energy Values (in kcal/mol Along with Standard Error) of the AAs with SO₄⁻ Water Molecules Obtained from Triplicate of MD Simulation

| S. no. | AA     | SO₄⁻²⁻ | Wat     | total    | average |
|--------|--------|--------|---------|----------|---------|
| 1      | HisP   | −24.82 ± 0.03 | −60.27 ± 0.02 | −85.09 ± 0.02 | −83.55 |
| Run1   | −26.74 ± 0.03 | −57.64 ± 0.01 | −84.38 ± 0.02 | |
| Run2   | −18.64 ± 0.02 | −62.56 ± 0.02 | −81.20 ± 0.02 | |
| Run3   | −1.27 ± 0.00 | −59.47 ± 0.02 | −60.74 ± 0.01 | −60.77 |
| HisN   | −1.56 ± 0.00 | −59.31 ± 0.02 | −60.88 ± 0.01 | |
| Run1   | −1.46 ± 0.00 | −59.25 ± 0.02 | −60.71 ± 0.01 | |
| Run2   | −0.55 ± 0.00 | −51.49 ± 0.02 | −51.74 ± 0.01 | −51.86 |
| Run3   | −0.31 ± 0.00 | −51.64 ± 0.02 | −51.96 ± 0.01 | |
| Phe    | −0.30 ± 0.00 | −51.60 ± 0.02 | −51.90 ± 0.01 | |
| Run1   | −1.32 ± 0.01 | −59.06 ± 0.02 | −60.38 ± 0.02 | −60.54 |
| Run2   | −1.29 ± 0.01 | −59.41 ± 0.02 | −60.71 ± 0.02 | |
| Run3   | −1.16 ± 0.00 | −59.37 ± 0.02 | −60.53 ± 0.01 | |
| Tyr    | −0.60 ± 0.01 | −60.16 ± 0.02 | −60.76 ± 0.02 | −60.67 |
| Run1   | −0.68 ± 0.01 | −59.74 ± 0.02 | −60.42 ± 0.02 | |
| Run2   | −0.88 ± 0.01 | −59.95 ± 0.02 | −60.84 ± 0.02 | |
| Run3   | −0.88 ± 0.01 | −59.95 ± 0.02 | −60.84 ± 0.02 | |

### Figure 4. Sum of electron density at the BCP (ρ(rₜ)) of the aromatic AA interaction with (A) SO₄⁻²⁻ and (B) water.

2.2. Atoms in Molecules Results. With the aid of Bader’s theory of atoms in molecules (AIM), it is possible to define the structure of the molecule/molecular complex quantum mechanically, and this indeed was widely used to characterize atom–atom interactions within molecular complexes in the case of non-covalent interactions. AIM analysis of the selected complexes was performed to understand the atomistic interaction within the whole molecular complexes and is presented in Figure 3. The electron density (ρ(rₜ)) and its Laplacian (∇²ρ(rₜ)) at the bond critical points (BCPs) of the interacting atoms in the case of SO₄⁻²⁻ and water molecules with the aromatic AAs were calculated, and their corresponding values are provided in Table 4. The AIM results are graphically illustrated in Figure 4. It is observed from the results that the sum of electron density at the BCPs decreases between the SO₄⁻²⁻ and aromatic AAs with the increase in water molecules, and on the other hand, the same increases between the water molecules and the aromatic AA system. The
results obtained illustrate that the water molecules play a vital role in stabilizing the divalent anion SO$_4^{2-}$ on the aromatic AA surface by coordinating with the anion and at the same time anchoring it to the $\pi$ surface of the aromatic AA, barring the case of tryptophan.

### 2.3. MD Simulation Results

The interaction energies of the anion and water with the aromatic AAs obtained from the MD simulation are presented in Table 5. It appears clear that the SO$_4^{2-}$ anion possesses a considerable interaction energy only in the case of HisP and that in the case of other AAs the interaction energy is very weak, whereas the water molecule possesses a stronger interaction energy with all the AAs. The observed order of interaction energies of the aromatic AAs in the SO$_4^{2-}$–water system is HisP > HisN, Trp, Tyr > Phe.

The results obtained from the simulation run in triplicate illustrate that the trend in the interaction energy remains the same and, furthermore, that the results are statistically significant. It is noteworthy to mention that the MD simulation also reproduces the results obtained from the quantum calculation with the HisP AA, exhibiting the strongest interaction with the solvated SO$_4^{2-}$, albeit in the cases of other AAs including HisN, Phe, Tyr, and Trp, we cannot observe results in line with the quantum results. The most possible reason for the abovementioned difference is the effect of temperature and the lack of force field sensitivity in handling such weak interactions. In the case of QC calculation, there is no temperature factor, that is, at 0 K in microsolvation, whereas in the case of MD simulation, the system is at macroscopic solvation at 300 K. This temperature factor and macroscopic versus microscopic systems may be responsible for the decrease in the magnitude of overall interaction energy in MD results compared to the QC results. Most importantly, the current force fields are parameterized well to treat the electrostatic and H-bond types of interactions, whereas the anion–$\pi$ kinds of weak interactions are not within the limit of the present force fields.

The interaction energy analysis carried out gave an estimate about the strength of the anion–water system interaction with the aromatic system, but it lacks any detail about the mode of interaction of anion and water around the aromatic AA.

![Figure 5. SDF of SO$_4^{2-}$ (yellow color) and water (red color) around the aromatic AAs derived from the MD simulation. The iso-surface value is $\sim$−0.07 (for SO$_4^{2-}$) and $\sim$0.07 (for water).](image)

| A.A  | SO$_4^{2-}$ | Wat+ SO$_4^{2-}$ |
|------|-------------|------------------|
| HisP | ![HisP SO4 2-](image) | ![HisP Wat+ SO4 2-](image) |
| HisN | ![HisN SO4 2-](image) | ![HisN Wat+ SO4 2-](image) |
| Phe  | ![Phe SO4 2-](image) | ![Phe Wat+ SO4 2-](image) |
| Tyr  | ![Tyr SO4 2-](image) | ![Tyr Wat+ SO4 2-](image) |
| Trp  | ![Trp SO4 2-](image) | ![Trp Wat+ SO4 2-](image) |
order to understand the mode of distribution of the SO$_4^{2-}$ and water molecules around the aromatic AA during the course of MD simulation, the spatial distribution of SO$_4^{2-}$ and water molecules around each AA was calculated (Figure 5). It is clear from the results obtained that there is a significant interaction of SO$_4^{2-}$ only in the case of HisP and Tyr. In the case of Tyr, the SO$_4^{2-}$ distribution is located around the $\pi$-OH group of the aromatic side chain, whereas in the case of HisP, the distribution of SO$_4^{2-}$ is observed around the circumference of the aromatic ring and on the top of the imidazole ring, which can be very well categorized as an anion–π interaction. The distribution of water molecules is observed in the case of all AAs. The spatial distribution function (SDF) analysis illustrates that the interaction of SO$_4^{2-}$ with the aromatic AA in the anion–π mode is only possible in the case of HisP, whereas in all other cases, it is not observed. The limitation of force fields should be taken into account while considering the SDF results.

Overall, the results obtained from the analysis of the QC calculations and MD simulations illustrate that divalent SO$_4^{2-}$ anions can be stabilized on the aromatic AAs only on solvation by water molecules and that among the aromatic AAs, only HisP can form anion–π interactions with significant interaction energy. It is to be noticed that analysis of the protein crystal structures from the PDB by Lucas and co-workers demonstrated the presence of anion–π interactions between the SO$_4^{2-}$ and His and Tyr. Our results show that the protonated histidine can form anion–π interactions with SO$_4^{2-}$ stabilized by the water molecules, but we cannot observe such a strong interaction of anion–π in the case of Tyr–SO$_4^{2-}$. The most possible reason for this discrepancy might be the temperature factor, as mentioned above. It is well known that during the crystallization process, the thermal perturbations are arrested, and the crystal formation takes place. Therefore, in such a scenario, the weak molecular interactions observed in the crystals may not be stabilized in the solution phase at room temperature. It is also important to note that most of the anion–π interactions are observed in the case of molecular crystals, where not only the temperature factors are absent but also the molecular packing effect can stabilize the weak molecular interactions. Recent studies on the improvement of force fields for cation–π and anion–ring interactions in proteins have also illustrated that these interactions are underestimated; specifically, the anion–ring interaction in the absence of a hydrogen bond is severely misjudged. In the case of solvent-mediated anion–π interactions, there is no direct hydrogen bonding between the anion and the π ring. The insufficiency of the current additive force fields in treating weak interactions like anion–π should be taken care in order to have a holistic understanding at the molecular level on biological processes.

3. CONCLUSIONS

It is obvious from the interaction energy values of the SO$_4^{2-}$–W$_n$ clusters with the aromatic AAs that solvation plays a crucial role in stabilizing the SO$_4^{2-}$ over the aromatic functionalities. The interaction strength of the AAs with the microlonsolvent SO$_4^{2-}$ was observed to be HisP > HisN > Tyr > Trp > Phe. The water molecules stabilize the SO$_4^{2-}$ over the aromatic AAs in the anion–π mode in the cases of HisP, HisN, Tyr, and Phe but not in the case of Trp. In spite of such observation that the anion–π interaction is stabilized in all aromatic AAs excluding Trp, the LE of the anion–π mode of interaction varies significantly among AAs. The protonated histidine possesses a very strong interaction with SO$_4^{2-}$–W$_n$ clusters, close to the covalent limit, whereas in the other cases, the interaction is in the weak non-covalent interaction limit. HisP requires at least three water molecules to stabilize the SO$_4^{2-}$ in an anion–π mode of interaction, whereas Phe requires five water molecules, and His and Tyr require eight molecules to stabilize such an anion–π mode of interaction. The electron density analysis from the AIM calculations shows the significant role of water molecules in stabilizing the SO$_4^{2-}$ anion on the surface of the aromatic AAs. The classical MD simulation of the aromatic AAs in the bulk solvation system shows the order of the interaction of SO$_4^{2-}$ to be HisP > HisN, Tyr, Trp > Phe. The spatial distribution analysis of the anion and water molecules around the aromatic AAs during the course of MD simulation illustrates that the anion–π mode of interaction between SO$_4^{2-}$ and AAs is only possible in the case of HisP at room temperature. The results obtained from this study provide a new understanding on the role of water molecules in stabilizing the anion–π interactions in biological systems, which will be useful in the areas of biological and biotechnological applications.

4. METHODOLOGY

4.1. DFT Calculations. In the DFT calculations, the aromatic groups protonated imidazole, imidazole, benzene, phenol, and indole (corresponding to the proteogenic AAs HisP, HisN, Phe, Tyr, and Trp, respectively) along with their beta carbons were considered as the model system for the aromatic AAs. The SO$_4^{2-}$ anion was shown to be solvated by 12 water molecules to completely saturate its solvation shell. Different systems with the AAs, the anion SO$_4^{2-}$ and different numbers of water molecules (W$_{1-12}$) were generated. The geometries of all the clusters were optimized using DFT with the M06-2X functional and employing the 6-31+G* basis set, which has been demonstrated to be most suitable for non-covalent interactions. Earlier reports revealed that this method is more reliable for the prediction of the structure and stability of the anion–π complexes in close agreement with CCSD(T) energies. Interaction energy (IE) of all clusters were calculated using the supermolecule approach and corrected for basis set superposition errors (BSSE) using the counterpoise (CP) procedure suggested by Boys and Bernardi. In the case of solvent-mediated anion–π interactions, there is no direct hydrogen bonding between the anion and the π ring. The insufficiency of the current additive force fields in treating weak interactions like anion–π should be taken care in order to have a holistic understanding at the molecular level on biological processes.
characterizing the different categories of weak and hydrogen-bonded molecular systems. All calculations were performed using the Gaussian 16 (revision A. 0.3) suite of programs and visualized using Gaussview.

4.2. MD Simulations. MD simulations were carried out with the five systems HisP, HisN, Phe, Trp, and Tyr with their ends capped with acetyl (ACE) and N-methylamine (NME) in order to avoid terminal charges, since we are here dealing with a charged solvent system consisting of SO₄⁻². The five AA systems were solvated with a box size of ~3.30 × 3.30 × 3.30 nm. The details of the number of ions and water molecules in each system are given in Table 6. The SO₄⁻² molecules were distributed throughout the entire box. The AMBER ff99SBILDN force field parameters were used for the AAs, SO₄⁻² was treated with parameters from Vila Verde and coworkers, and water molecules were treated with the TIP3P water model. All simulations were carried out with periodic boundary conditions. The minimization of the initial geometries was carried out using the steepest descent algorithm followed by an equilibration step for 250 ps in NPT followed by another equilibration of 500 ps in the NPT ensemble (temperature gradually increased to 300 K). The temperature was retained at 300 K using the stochastic velocity rescaling method as described by Bussi, and pressure was maintained at 1 bar using a Nose-Hoover barostat. A 2 fs time step was used to integrate the equation of motion, as it was found to be suitable for such a small system and TIP3P water molecules by Lindorff-Larsen et al. The electrostatic interaction was calculated using particle mesh Ewald sums with a non-bonded cutoff distance of 10 Å. A non-bonded cutoff of 10 Å was used. Bonds between hydrogen and heavy atoms were constrained at their equilibrium values using the LINCS algorithm. Analysis of the energy parameters revealed that the systems were well equilibrated. Subsequently, each system was subjected to a production run of 100 ns with trajectories being saved every 1 ps for further analysis. The MD simulation runs were carried out in triplicate for the sake of statistical significance. The production run and analysis of the trajectories were carried out using the GROMACS 2018.3 suite of programs, and the results were visualized using the VMD package. The interaction energies between the AAs, SO₄⁻² and water molecules were calculated throughout the trajectory. The energy groups such as proteins, SO₄⁻², and water molecules are defined in the MD input, and the corresponding energy files are generated at an interval of 2 fs. The pairwise interaction energies from the whole trajectory were calculated using the gmx energy analysis. The SDF of the SO₄⁻² and water molecules around the AAs was calculated from the MD simulation of individual AAs in SO₄⁻²—water medium.

Table 6. Details of the Study Systems for MD Calculations

| AA  | box size (nm) | SO₄⁻² | Na⁺ | Wat |
|-----|--------------|-------|-----|-----|
| HisP | 3.3 × 3.3 × 3.3 | 30 | 59 | 1000 |
| HisN | 3.3 × 3.3 × 3.3 | 30 | 60 | 999 |
| Phe  | 3.3 × 3.3 × 3.3 | 30 | 60 | 988 |
| Tyr  | 3.3 × 3.3 × 3.3 | 30 | 60 | 996 |
| Trp  | 3.3 × 3.3 × 3.3 | 30 | 60 | 1006 |

Table 6. Details of the Study Systems for MD Calculations

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**Notes**

The authors declare no competing financial interest.

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