Understanding proton affinity of tyrosine sidechain in hydrophobic confinement[#]

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Abstract. Tyrosine is an important amino acid residue that plays a key role in several biochemical transformations such as, abstraction/donation of proton from/by its sidechain. We present here a density functional study on the proton affinity of tyrosine sidechain suspended inside the core of a single walled carbon nanotube that mimics the environment of protein structural pores and molecular channels. Tyrosine is found to exhibit a lower reactivity on confinement and unlike several other polar amino acid sidechains, its reactivity does not respond to hydrogen bonding with neighbouring hydroxyl groups.

Keywords. Tyrosine; proton affinity; carbon nanotube.

1. Introduction

The specific function of an enzyme often requires polar amino acid sidechains as key reactants at the active site or as mediators in the transport of ions through molecular channels.¹ For example, a proton may be transported through a predominantly non-polar channel of an enzyme via hydrogen bonded networks formed by polar amino acid sidechains and water molecules. The protonation state of the amino acid sidechain in all these cases turns out to be a crucial factor in determining the underlying mechanism.² However, in the presence of several amino acid sidechains with suitable protonation states, several competing pathways may appear plausible and their relative efficiencies in the overall mechanism need to be evaluated. For any such evaluation, it may be required to compare relevant reactivity parameters of competing sidechains when present, for example, confined within a buried active site as opposed to being exposed to the bulk solvent. In this article, we investigate the effect of confinement on the reactivity of tyrosine sidechain and compare its behaviour to the known properties of other polar amino acid sidechains in similar environment.

Tyrosine (Tyr), like histidine (His), is known to play a crucial role in a wide range of chemical and biochemical processes³ primarily through (a) deprotonation of the phenolic –OH group present in its sidechain and

(b) formation of hydrogen bond in both protonated and deprotonated states of the sidechain. The importance of Tyr in enzyme catalysis is exemplified, for instance, by carboxypeptidase A, an enzyme belonging to the family of exopeptidases, containing one zinc ion bound to a single polypeptide chain of 307 amino acids. Its biological function is to catalyze the hydrolysis of C-terminal amino acids from polypeptide substrates and it exhibits a preference toward those substrates possessing large, hydrophobic C-terminal sidechains. Several studies indicate the role of a tyrosine residue, Tyr-248, at the active site of the protein in stabilizing the substrate and in forming a hydrogen bond to the scissile peptide bond after rotation from the protein surface to the active site.⁴ It has also been suggested that phenolic hydroxyl of Tyr-248 probably acts as a proton donor to the NH of the peptide bond and not as a nucleophile.⁴ In the well studied enzyme human carbonic anhydrase II (HCA II), a crucial role is played by a tyrosine residue, Tyr-7, in the active site, not only in providing a translocation path for an excess proton, but also in stabilizing the fluctuating conformations of a neighbouring His residue that is widely believed to be a proton shuttle between the active site and the bulk solvent.⁵ Although most of the current analyses of HCA II mechanism are based on Tyr-7 sidechain being in its protonated state, recent neutron diffraction studies seem to indicate that Tyr-7 may be present in the active site of HCA II with its sidechain in an unprotonated phenoxide form.⁶ Needless to say, any such alteration of the protonation state of Tyr-7 sidechain would crucially affect the free energetics of the alternative pathways that have been proposed to supplement the His-mediated

[#]Dedicated to Prof. N Sathyamurthy on his 60th birthday

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shuttle to exchange proton between the active site and the protein surface.

It is therefore evident from the above discussion that the participation of Tyr in a given step of an enzymatic catalysis would depend on the proton donation or uptake by its sidechain within the confined environment of the active site. Ideally one would set up a fully quantum mechanical estimation of proton affinity of Tyr sidechain in the enzymes cited earlier. However, on account of the complexity of the systems described here, both in terms of structure and dynamics, a simpler model system may serve as a better starting point for the analysis. In the present investigation, we use a single walled carbon nanotube (SWCNT) to mimic a hydrophobic channel at a molecular level. Confinement in this hydrophobic environment may significantly affect properties such as reactivity parameters and thus control the outcome of simple chemical reactions that take place in it. In a recent study using density functional methods, we have reported in detail the variation of proton affinities of several polar amino acid sidechains, suspended inside the SWCNT.¹ It has been found that for residues such as His, Asp, Glu, Ser and Thr, a marked increase in proton affinity is observed if these sidechains are restricted within the hydrophobic channel in the presence of hydrogen bonding with neighbouring polar groups. In the present work, we extend this study to investigate the proton affinity of tyrosine sidechain when it is suspended from the inner wall of a SWCNT.

The gas phase proton affinity (PA) of an amino acid sidechain suspended inside CNT can be defined as the negative of the reaction enthalpy ($-\Delta H$) of following protonation reaction

Here $\Delta H = H(BH^+) - H(B)$ corresponds to the enthalpy of protonation of the base, B and is calculated as¹ $\Delta H = \Delta E + \Delta ZPVE$ where ΔE and $\Delta ZPVE$ denote the changes in ground state electronic energy and zero point vibrational energy of protonated and unprotonated forms, respectively. We present the results of our calculations of sidechain proton affinity of tyrosine using density functional theory method in this article. The effect of hydrophobic confinement is estimated by comparing with the corresponding value calculated in the gas phase. It may be noted that experimental studies on proton affinity of amino acids mostly report the proton affinity corresponding to the $-NH_2$ group of the peptide back bone.⁷ However, in the present context, we focus on exploring the sidechain proton affinity only. Our results reveal that Tyr sidechain exhibits a lower

proton affinity and less sensitivity towards fluctuations in polarity within hydrophobic channels compared to other amino acid residues with polar sidechains.

2. Method

A brief outline of the computational method employed to calculate the proton affinity of an amino acid sidechain within SWCNT¹ is presented below. Further details are available in reference Abi et al.¹

The very first step in our calculations involves generation of the carbon nanotube structure functionalized with an amino acid sidechain suspended from the inner wall and extending into the core of the tube. We have constructed an armed chair 6:6 SWCNT of diameter 8.14 Å and length 13.0 Å using the utilities of Dmol³.⁸ We modified one sp^2 -hybridized C-atom of the CNT into sp^3 -hybridized one and suspended the Tyr sidechain from this modified C-atom into the core of the tube. The modified C-atom thus serves as the C_α atom of Tyr sidechain. In order to understand the effect of hydrogen bonding inside CNT, we further modified the model by introducing five polar $-OH$ groups suspended from the wall of the tube at a distance of 3.5 Å from the sidechain of the amino acid. In each case, care was taken to ensure that no segment of Tyr sidechain or $-OH$ groups protrude out of the channel and all these groups remain suspended well within the hydrophobic core of the tube. It may be noted that in the present work, we have used a CNT that is slightly longer than that used in our previous work (with a length of 9.84 Å) in order to confine the entire length of Tyr within the tube. The resultant structures, optimized using PWC-LDA⁹ density functional with minimal basis set, have been shown in figure 1 corresponding to both cases, the absence and presence of neighbouring polar $-OH$ groups.

Calculations of single point and zero point vibrational energies of the optimized structures were carried out using the BLYP/dnp¹⁰ method in Dmol³.⁸ The method used in our work has already been calibrated using the known sidechain proton affinity of His and discussed in our previous study.¹ The enthalpy of protonation of the base (ΔH) (and the proton affinity) is calculated from the energy values thus obtained. In the rest of the article, we shall refer to the structures without and with 5 $-OH$ groups, respectively, suspended from the wall of the SWCNT as models 1 and 2.

3. Results and discussion

In this section, we shall present the results of our calculations on the model systems discussed above. We

Figure 1. Optimized structures of (a,c) unprotonated, (b,d) protonated sidechains of Tyr suspended from the wall inside a single walled carbon nanotube (a,b) in the absence and (c,d) in the presence of 5 -OH groups within hydrogen bonding distance from the phenolic oxygen of the Tyr sidechain.

first carried out the computation of proton affinity of Tyr sidechain attached to a methyl group ($\text{CH}_3\text{-CH}_2\text{-Ph-OH}$) in the gas phase. This structure serves as simplest gas phase analogue of the Tyr sidechain ($\text{-CH}_2\text{-Ph-OH}$) that is linked to the modified C-atom on the inner wall of the CNT in models 1 and 2. As shown in table 1, the proton affinity of the Tyr sidechain analogue is found to be $358.07 \text{ kcal mol}^{-1}$. We have also examined the case where the C_α -atom of Tyr sidechain is linked to -NH_2 and -COOH groups. The corresponding value of proton affinity ($351.59 \text{ kcal mol}^{-1}$) does not exhibit any marked change in the presence of the *peptide linkage*. The gas phase proton affinity value of Tyr backbone has been reported to be $220.9 \text{ kcal mol}^{-1}$ from experimental studies⁷ and $223.5 \text{ kcal mol}^{-1}$ from density functional based B3LYP/6-31G(d,p) calculations.¹¹ Interestingly,

both these values are substantially lower than those predicted by our calculations for the sidechain in the model gas phase analogue. In table 1, we have presented

Table 1. Estimation of gas phase proton affinity (PA) values of selected amino acid sidechains connected to a methyl group instead of a peptide backbone.

Sidechain	$-\Delta E$ kcal mol^{-1}	ΔZPVE kcal mol^{-1}	$PA = -\Delta H$ kcal mol^{-1}
Tyr	367.16	9.09	358.07
Asp	359.24	8.33	350.91
Ser	399.01	8.36	390.65
His	245.20	7.77	237.43

Table 2. Proton affinity (PA) values of Tyr sidechain in the gas phase and confined inside a single-walled carbon nanotube (CNT). Models 1 and 2 for the CNT represent the Tyr sidechain present within CNT in the absence and in the presence of neighbouring polar OH groups, respectively. All energy values reported in the table estimated using the BLYP/dnp method.

Medium	$-\Delta E$ kcal mol ⁻¹	$\Delta ZPVE$ kcal mol ⁻¹	$PA = -\Delta H$ kcal mol ⁻¹
Gaseous phase	367.16	9.09	358.07
CNT Model 1	341.91	7.71	334.20
Model 2	333.39	5.24	328.15

the proton affinity values for gas phase analogues of the sidechains in Asp, His and Ser. Gas phase proton affinity of Tyr is found to be in between those predicted for the analogues of Asp and Ser and is much higher compared to that of His.

We have presented in table 2 the variations in ΔE , $\Delta ZPVE$ and PA as we modify the sidechain inside the SWCNT as per models 1 and 2. As expected in model 1, deviations from gas phase values are observed in both ΔE and $\Delta ZPVE$, the latter recording a relatively higher variation. It may be noted that in order to facilitate a comparison between the values reported here and those obtained earlier, we have used the same diameter of 8.14 Å for the CNT. This creates considerable difficulty in optimizing the structure when the long Tyr sidechain is restricted inside the CNT. We have shown here results from the optimized structure where the sidechain remains sheltered in the core of the tube and the phenolic ring retains its planarity. The proton affinity of Tyr sidechain under such circumstances is found to decrease inside the hydrophobic core. This may be rationalized by noting that the protonated form of Tyr sidechain is neutral while the unprotonated form is negatively charged. Therefore, uptake of a proton inside the CNT is expected to be favourable compared to retaining the anionic sidechain. Similar decrease in proton affinity values on transfer from gas phase to the CNT has been observed in the case of Glu, Ser and Thr sidechains,¹ all associated with neutral protonated and anionic unprotonated forms.

Perhaps the most unexpected behaviour is encountered as we investigate the results from model 2. In the presence of five additional –OH groups, all suspended within hydrogen bonding distance from the sidechain –OH group of Tyr, a marginal decrease in the proton affinity value is observed. This is in striking contrast to the marked increase of more than 100 kcal mol⁻¹ that has been noted especially in the case of Ser and Thr under similar conditions. Even Asp and Glu sidechains

were reported to recover their gas phase proton affinities under these model conditions. However, this may be explained as follows, the Tyr sidechain is not perpendicular to the tube axis in the optimized structure and its bent configuration contributes little to accommodate the additional –OH groups within the restricted space. In fact, we repeatedly failed in our attempts to optimize structures where the –OH groups are chosen at locations preventing the formation of any hydrogen bond with the Tyr sidechain. This observation indicates a subtle balance between the stabilization derived through hydrogen bonding interactions between Tyr sidechain and the –OH groups and the destabilization through a steric crowding of the core when all the suspended groups lie close to each other.

4. Conclusion

The calculated values of sidechain proton affinity of Tyr being positive, it follows that the protonated sidechain will be much more stable compared to the deprotonated one under hydrophobic confinement. This would probably indicate the presence of a neutral sidechain of Tyr-7 that is quite well packed at the active site of HCA II. The anionic form may be favoured in the cases where point mutations seriously deplete the extensive water network otherwise spanning the active site cavity of HCA II. In the case of carboxypeptidase A, rotation of Tyr-248 sidechain brings it to a more crowded environment of the active site cavity and correspondingly, proton donation by the sidechain to the peptide bond seems favourable in comparison to a nucleophilic attack by the corresponding deprotonated form. However, more detailed studies to include mapping of the active site cavity in terms of size and hydrogen bonding interactions would be needed to derive any quantitative conclusion. The remarkable increment of sidechain proton affinity, as observed for Ser and Thr in the presence of hydrogen bonding interactions with neighbouring polar groups, has not been observed here. However, increasing the dimensions of the molecular channel may decrease the steric destabilization so as to allow the effects of hydrogen bonding to become noticeable. The research work is in progress in this direction.

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