High resolution X-ray emission: carbonic anhydrase solvent protonation

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ABSTRACT Numerous studies have been performed on carbonic anhydrase (CA) in order to determine the protonation state of the solvent molecule bound to Zn. Recent Zn NMR studies [Lipton et al 2004] of CA demonstrated that there is no change in the Zn structure, as detected by ^{67}Zn-NMR, as the pH is changed from 5-9. This is in contrast with the prevailing mechanisms for explaining the pH dependence of CA activity, and suggests that the site of protonation is distant from the Zn-bound solvent molecule. We have recently performed high resolution X-ray emission spectroscopy (XES) on CA and on two models mimicking, respectively, Zn-OH and Zn-OH_2 [Bergquist et al 2003]. A peak in the emission spectra at the low energy side of the $K\beta_{2,5}$ has been attributed to a ligand 2s to metal 1s transition. The intensity and energy of this so called "cross-over" peak is sensitive to the details of the ligation. We find that the crossover peak for the aquo model complex occurs at a lower energy than that for the hydroxo complex, consistent with the stabilization that is expected on protonation of the solvent. DFT and XES calculations (FEFF) for the model compounds show good agreement with the measured XES data for both the aquo and hydroxo models. For CA, spectra measured at pH 5.5 and 8.5 follow a trend similar to that seen for the model compounds, suggesting that, from the perspective of XES, the Zn site *does* change with pH. We are currently exploring the interpretation of these results in terms of the protonation state of the solvent, the local electrostatic charge of the Zn site, and changes of the electric field gradient that occur as a consequence of protonation

References

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