Mechanistic Aspects of Nitrogen Cycle: the Action of Copper-Containing Nitrite Reductase

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The nitrogen cycle is a natural process in which nitrogen is converted between biological and non-biological forms. Nitrogen fixation and nitrification convert nitrogen gas into nitrate, while denitrification converts nitrate back into nitrogen gas. The balance between nitrification and denitrification has been altered by human activities of using synthetic fertilizers since the discovery of the Haber-Bosch process. As a result, the excess nitrogen in biosphere has caused many environmental problems such as eutrophication and harmful algal bloom. Denitrification is currently the only proven nitrogen removing process, which is catalyzed by complex metalloenzymes with transition metal cofactors. Copper-containing nitrite reductase (CuNiR) performs a key step in denitrification by catalyzing the reduction of NO_2^- to NO. Experimental X-ray data have provided valuable insight into the overall function of CuNiR. However, many important questions remain unanswered. We have performed a computational study of the enzymatic mechanism of CuNiR based on density functional theory. Our results determine the minimum energy pathways, transition states and the activation energy barriers of each step in the reaction. A critical residue Asp^{98} is found to stabilize the initial attachment of nitrite. It also contributes to stability of a previously reported "side-on" coordination of the nitrosyl intermediate, although this geometry does not occur during the reaction. We also find that the transformation of the O- to N-attachment is achieved by an electron transfer from Type I copper.