# Enzymatic "Tricks": Carboxylate Shift and Sulfur Shift

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# Enzymatic "Tricks": Carboxylate Shift and Sulfur Shift

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We look into two interesting phenomena that occur in enzymes: one has been termed carboxylate shift and the other sulfur shift. Carboxylate shift is an interesting mechanistic phenomenon, which is characterized by a change in the coordination mode of a carboxylate group (monodentate to bidentate or vice versa) with ligand entrance or ligand exit from the metal coordination sphere. We focus on some patterns relating the intrinsic characteristics of a given metal coordination sphere to the occurrence of a carboxylate shift and the corresponding energy stabilization suffered by the enzyme. A structural rearrangement known as sulfur shift has been recognized to occur in some Mo-containing enzymes of the DMSO reductase family. This mechanism is characterized by the displacement of a coordinating cysteine thiol or selenocysteine thiol from the first to the second shell of the Mo-coordination sphere metal, allowing for direct coordination of the incoming ligand (substrate or inhibitor) to the hexacoordinated Mo ion found in the enzyme unbound state. The sulfur shift, as well as the carboxylate shift, results in a rearrangement of the enzymatic catalytic center that provides an efficient mechanism to keep a constant coordination number throughout an entire catalytic pathway. © 2014 Wiley Periodicals, Inc.

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## Introduction

Enzymes are amazing molecular machines with intrinsic and intricate catalytic mechanisms in which only a small region, the active center, participates actively in the reaction cycle. The remaining structure works as a modulator of those active centers, either stabilizing or destabilizing the existing stationary states along the potential energy surfaces.[1–3] In this study, we analyze two interesting mechanistic phenomena; two "tricks" used by metalloproteins to efficiently keep constant their coordination number throughout their entire catalytic pathway. Accordingly, here we look into two interesting phenomena from an enzymatic point of view—one has been termed carboxylate shift[4] and the other sulfur shift.[5] Figure 1 shows a schematic representation of both the carboxylate shift and the sulfur shift using zinc and molybdenum as examples of coordinating metals.

Some studies have been conducted for carboxylate shift as regards to zinc[6] in enzymes such as farnesyltransferase[7,8] and thermolysin (TLN)[9] enzymes. Features such as the nature and identity of the ligands in the metal coordination sphere, their relationship with the existence of a carboxylate-shift mechanism, and the Gibbs activation and reaction energies for ligand entrance and ligand exit were the subject of particular attention in some of those studies.

Conversely, the establishment of what has been termed sulfur shift,[5] partly because of its similarity to carboxylate shift, is very recent and few studies have been conducted to unravel trends and patterns relating these features to the intrinsic characteristics of a given metal coordination sphere. At the moment, molybdenum is the only metal that has been linked to the sulfur shift. Studies directed at molybdenum have been carried out to elucidate on the catalytic mechanism of molybdenum-containing enzymes.[10] However, whether we consider carboxylate shifts or sulfur shifts, these occurrences seem to exist as a way of nature to keep enzymatic energetic cost low.

In this study, we aim at describing what are carboxylate shifts as well as sulfur shifts, when do they turn up and how they contribute to enzymatic catalytic mechanisms.

## Carboxylate Shift

Both glutamic and aspartic acids coordinate through their carboxylate side chains, displaying identical $pK_a$ values. They can coordinate the metal ion through the oxygen atoms, either in a monodentate or a bidendate way. In metalloenzymes, a carboxylate shift is characterized by a change in the coordination form of a carboxylate group to the metal in the enzyme. This change can occur either in mononuclear or polynuclear systems with basically identical outcome, albeit in the latter these changes can take far more complex forms.[11] This type of phenomenon has been observed in a number of model compounds containing Fe,[12–14] Mn,[14–16] Cd,[17] and Zn.[11]

In mononuclear metalloenzymes, the coordination to the metal changes typically from monodentate to bidentate (Fig. 1) with ligand exit, or vice versa with ligand entrance. The ability of the carboxylate group to rearrange in such a manner provides a rather remarkable example of a metalloenzyme

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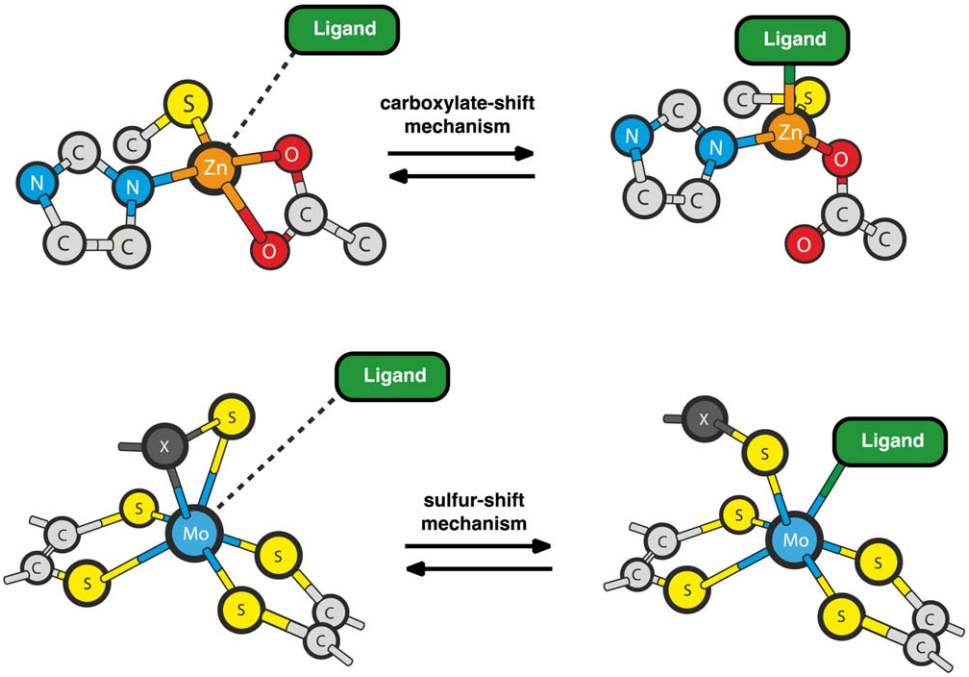


**Figure 1.** Schematic representation of the carboxylate shift, found in farnesyltransferase (top), and sulfur-shift mechanism (bottom) found in nitrate reductase (X = Cys) and formate dehydrogenase (X = SeCys). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

maintaining a constant or nearly constant coordination number throughout its catalytic pathway and, consequently, lowering the activation barriers for ligand entrance or exit. Such is the case of some Zn centers for which the differences in Gibbs activation barriers for different ligands' exit have been determined[6] both with a free and a frozen carboxylate group (i.e., one in which the larger metal–carboxylate oxygen distance was kept constrained to its value in the optimized monodentated state, Fig. 2), thus mimicking, respectively, the possibility versus the impossibility of a carboxylate shift to occur.

The calculations also show that the magnitude of such an effect depends on the exact combination of amino acid residues at the metal coordination sphere and to a lesser extent on the identity of the ligand to be exchanged. Obviously, in biological systems, these aspects will be further influenced by the specific enzymatic environment surrounding each metallic center.

Does this mean that all zinc-enzymes or other metalloenzymes will undergo carboxylate shift whenever the necessary conditions are fulfilled, that is, whenever glutamatic/aspartic acids are coordinated to a metal in the active center of an enzyme? Apparently not! Zhang and coworkers[9] have performed hybrid QM/MM (quantum mechanics/molecular mechanics) molecular dynamic simulations on TLN and histone deacetylase 8 (HDAC8) and have observed different coordination modes and fast ligand exchange on both enzymes. However, whereas in TLN carboxylate shift is thought to occur, in HDAC8 only the monodentate binding mode for the carboxylate coordination was observed.

Factors that seem to govern the carboxylate-binding mode (monodentate vs bidentate) in metalloproteins, and, therefore, the carboxylate-shift phenomenon have been mentioned in the literature.[4,18,19] Accordingly, bidentate carboxylate binding is preferred over the monodentate mode under the following conditions: (1) high-coordination number of the metal cation; (2) charge from the second carboxylate oxygen easily accepted by the metal cation, which displays, thus, good acid Lewis properties; (3) the positive charge of the metal cation is not neutralized by charge transfer from negatively charged ligands in the metal complex; (4) the metal cation is relatively large, easily accommodating bulky protein main chain/side chain dipoles; and (5) the first- or second-shell ligands are poor or nonexistent hydrogen bond donors. Moreover, from the data gathered, they concluded that a carboxylate monodentate-bidentate switch, together with other structural factors, can be used to fine tune the metal-binding site affinity and/or selectivity; this translates into a modification of the function/properties of corresponding metalloproteins.

## Sulfur Shift

The sulfur-shift mechanism has been defined as a molybdenum coordination change involving a first to second shell displacement (shift) of one of the metal's ligands (a sulfur or a selenium atom), resulting in a free coordination position that is used, by the enzyme, to bind the substrate with a low-energetic cost. This phenomenon has been observed in molybdenum-metalloenzymes from the dimethyl sulfoxide reductase (DMSOr) family[5,10,20,21] but in none others till now, to the best of our knowledge.

The DMSOr family comprises Mo-enzymes of prokaryotic origin exclusively. Enzymes from this family present a hexacoordinated Mo ion bound to four sulfur atoms from two dithiolene moieties of two pyranopterin molecules and two extra ligands,

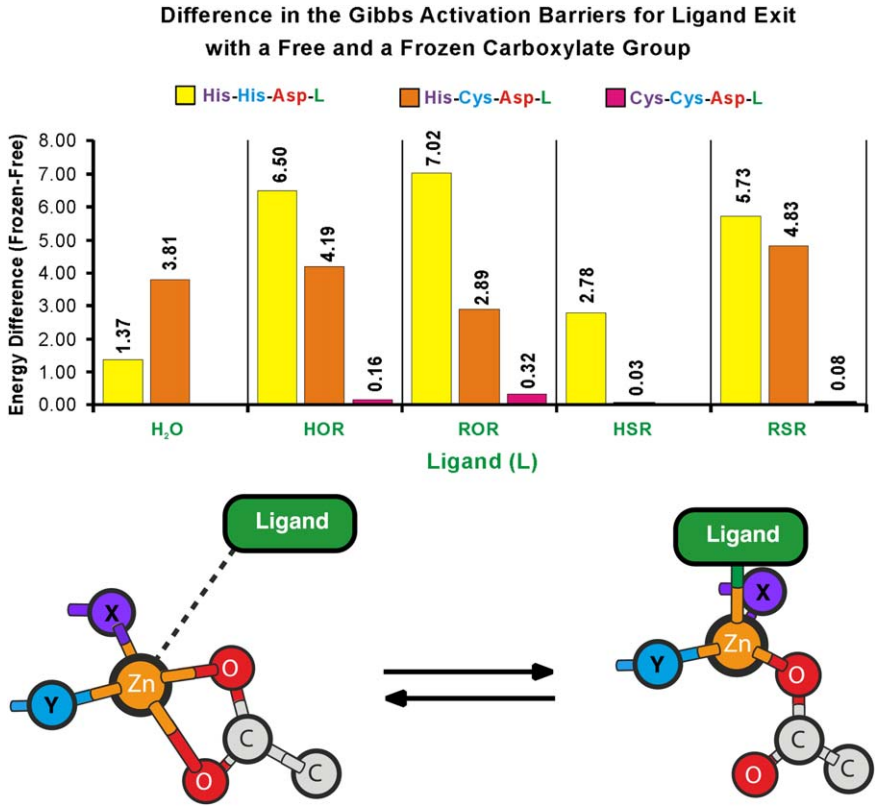


**Figure 2.** Difference in the Gibbs activation energies (kcal/mol) for ligand exit with a free and a frozen carboxylate group (i.e., with the second metal-carboxylate bond kept constrained to its value in the optimized monodentated state) for the several coordination spheres and ligands studied. R corresponds to a $CH_3$ group. Gibbs energies approximately calculated in the frozen carboxylate results [data taken from Ref. 6]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

which are highly variable. The sulfur-shift mechanism occurs in the enzymes in which the fifth coordination position to the Mo ion is occupied by one sulfur atom from a cysteine residue (or selenium from SeCys) while one inorganic sulfur ligand completes the sixth position. This subfamily comprises periplasmic nitrate reductases (Nap), Fdh, and polysulfide reductases (Psr).[22]

Calculations performed at density functional theory on nitrate reductase and formate dehydrogenase (Fig. 3) have shown that the sulfur-shift mechanism results from a combination of three simultaneous primitive changes[5,20,21]: (1) movement of Cys140 in Nap and SeCys140 in Fdh from the first shell to the second shell of the molybdenum ion, (2) change of the coordination position of the sulfido to the place previously occupied by the

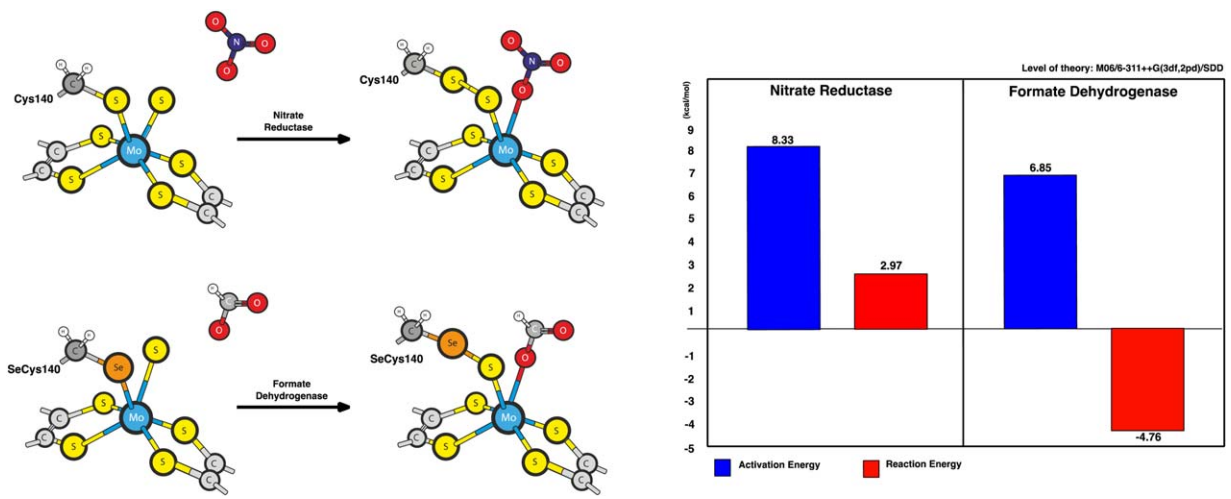


**Figure 3.** Left: The sulfur-shift mechanism in nitrate reductase (Nap) and in formate dehydrogenase (Fdh). Right: Activation and reaction free energies involved in the sulfur-shift mechanism of Nap and Fdh. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

residue, and (3) coordination of the substrates ($NO_3^-$ in Nap and $HCO_2^-$ in FdH) to the Mo ion in the opened coordination position. This mechanism allows, therefore, that both enzymes can exchange easily between active and inactive forms, and maintain the hexacoordination of the metal throughout the entire catalytic pathway. Consequently, this mechanism lowers the enzymes activation energies for ligand entrance or exit.

The sulfur-shift mechanism is also in line with other mechanistic proposals.[23] For instance, Hofmann showed that nitrate reduction with a persulfo molybdenum complex fragment is favored over the oxidation of a molybdenum-bound sulfur atom.[24] Xie et al. also showed that this type of mechanism is feasible under physiological conditions.[25] The same authors have proposed another type of mechanism for substrate reduction too, in which the substrate binds directly to the Mo ion and forms a heptacoordinated complex. Although a better energetic profile for this reaction was obtained, these reactions were modeled without the inclusion of the protein residues that surround the metal complex, a condition that might preclude the formation of the heptacoordinated complex and are detrimental for the sulfur-shift mechanism observed in Nap and FdH.[5] Another factor that supports the sulfur-shift mechanism is recent experimental data that reveals interesting changes close to the molybdenum active site, suggesting a movement of the coordinating Cys. This result supports a reaction mechanism, involving the rearrangement of the coordinating Cys, which enables a substrate coordination position at the Mo ion, that is, the sulfur-shift mechanism.[26]

## Conclusions

Both, the already more established carboxylate shift and the more recently proposed sulfur-shift mechanism are important phenomena, "tricks," that enzymes perform and allows them to undergo a fast and controlled process of ligand exchange dependent on an alteration in the metal coordination sphere that lowers the Gibbs activation barrier for the process. However, particular combinations of residues and a generic carboxylate ligand (Glu or Asp) result in different magnitudes for the respective carboxylate-shift processes. In fact, this seems to be more important for the magnitude of the effect than the identity of the ligand to be exchanged.

The recently established sulfur-shift mechanism is very similar to the carboxylate-shift mechanism that is found in many metalloenzymes. Both the mechanisms present an efficient way for ligand entrance or ligand exit, while the metal ions maintain a constant coordination number through the catalytic cycle. The main difference between both mechanisms seems to be the fact that the sulfur-shift mechanism involves the change in the coordination of a sulfur atom from Cys (or Se-Cys), moving from the first to the second shell, while the carboxylate shift involves a monodentate/bidentate exchange of a carboxylate group within the first shell. The energetic profiles of both mechanisms are very similar, allowing the enzymes to rapidly interchange between the two forms with low-activation barriers and almost thermoneutral reactions. This type of phenomena seems to be a way that enzymes adopt to control the access of non-substrate molecules to metal cofactors.

**Keywords:** carboxylate shift · sulfur shift · catalytic mechanism · farnesyltransferase · periplasmic nitrate reductase · formate dehydrogenase

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[1] S. F. Sousa, P. A. Fernandes, M. J. Ramos, *Phys. Chem. Chem. Phys.* **2012**, *14*, 12431.
[2] M. J. Ramos, P. A. Fernandes, *Acc. Chem. Res.* **2008**, *41*, 689.
[3] N. M. F. S. A. Cerqueira, P. A. Fernandes, M. J. Ramos, *J. Chem. Theory Comput.* **2011**, *7*, 1356.
[4] R. L. Rardin, W. B. Tolman, S. J. Lippard, *New J. Chem.* **1991**, *15*, 417.
[5] N. M. F. S. A. Cerqueira, P. A. Fernandes, P. J. Gonzalez, J. J. G. Moura, M. J. Ramos, *Inorg. Chem.* **2013**, *52*, 10766.
[6] S. F. Sousa, P. A. Fernandes, M. J. Ramos, *J. Am. Chem. Soc.* **2007**, *129*, 1378.
[7] S. F. Sousa, P. A. Fernandes, M. J. Ramos, *Biophys. J.* **2005**, *88*, 483.
[8] S. F. Sousa, P. A. Fernandes, M. J. Ramos, *Chem. Eur. J.* **2009**, *15*, 4243.
[9] R. B. Wu, P. Hu, S. L. Wang, Z. X. Cao, Y. K. Zhang, *J. Chem. Theory Comput.* **2010**, *6*, 337.
[10] S. Najmudin, P. J. Gonzalez, J. Trincao, C. Coelho, A. Mukhopadhyay, N. M. F. S. A. Cerqueira, C. C. Romao, I. Moura, J. J. G. Moura, C. D. Brondino, M. J. Romao, *J. Biol. Inorg. Chem.* **2008**, *13*, 737.
[11] A. Demsar, J. Kosmrlj, S. Petricek, *J. Am. Chem. Soc.* **2002**, *124*, 3951.
[12] J. Kuzelka, B. Spingler, S. J. Lippard, *Inorg. Chem. Acta* **2002**, *337*, 212.
[13] G. Lemercier, E. Mulliez, C. Brouca-Cabarrecq, F. Dahan, J. P. Tuchagues, *Inorg. Chem.* **2004**, *43*, 2105.
[14] R. L. Rardin, A. Bino, P. Poganiuch, W. B. Tolman, S. C. Liu, S. J. Lippard, *Angew. Chem. Int. Ed.* **1990**, *29*, 812.
[15] C. Baffert, M. N. Collomb, A. Deronzier, S. Kjaergaard-Knudsen, J. M. Latour, K. H. Lund, C. J. McKenzie, M. Mortensen, L. Nielsen, N. Thorup, *Dalton Trans.* **2003**, *9*, 1765.
[16] D. Pursche, M. U. Triller, N. Reddig, A. Rompel, B. Krebs, Z. Anorg. *Allg. Chem.* **2003**, *629*, 24.
[17] S. L. Zheng, W. Li, G. Yang, H. G. Zhu, B. H. Ye, X. M. Chen, *Aust. J. Chem.* **2002**, *55*, 611.
[18] T. Dudev, C. Lim, *Acc. Chem. Res.* **2007**, *40*, 85.
[19] B. F. Gherman, M. H. Baik, S. J. Lippard, R. A. Friesner, *J. Am. Chem. Soc.* **2004**, *126*, 2978.
[20] N. M. F. S. A. Cerqueira, P. J. Gonzalez, C. D. Brondino, M. J. Romao, C. C. Romao, I. Moura, J. J. G. Moura, *J. Comput. Chem.* **2009**, *30*, 2466.
[21] C. S. Mota, M. G. Rivas, C. D. Brondino, I. Moura, J. J. G. Moura, P. J. Gonzalez, N. M. F. S. A. Cerqueira, *J. Biol. Inorg. Chem.* **2011**, *16*, 1255.
[22] P. J. Gonzalez, M. G. Rivas, C. S. Mota, C. D. Brondino, I. Moura, J. J. G. Moura, *Coord. Chem. Rev.* **2013**, *257*, 315.
[23] S. Metz, W. Thiel, *Coord. Chem. Rev.* **2011**, *255*, 1085.
[24] M. Hofmann, *J. Biol. Inorg. Chem.* **2009**, *14*, 1023.
[25] H. J. Xie, Z. X. Cao, *Organometallics* **2010**, *29*, 436.
[26] C. Coelho, P. J. Gonzalez, J. J. G. Moura, I. Moura, J. Trincao, M. J. Romao, *J. Mol. Biol.* **2011**, *408*, 932.

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