

# (Salen)Mn(III) Compound as a Nonpeptidyl Mimic of Catalase: Theoretical Study of the Reaction Mechanism and Comparison with (Salen)Mn Catalyzed Epoxidation Activities.

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## ABSTRACT

Catalase mimics can be used as therapeutic agents against oxidative stress in treatment of many diseases including Alzheimer's disease, stroke, heart disease, aging and cancer. (Salen)Mn(III) compounds have been proven to be promising as synthetic antioxidants that, in particular, dismutate  $H_2O_2$  resulting in two water molecules and oxygen. For the first time we have performed quantum chemical investigation of catalase activity of (salen)Mn(III) biomimetic compound using the DFT method. A ping-pong mechanism approach has been considered to describe the dismutation reaction. Part I of the reaction –oxidation of the metal atom of the catalyst–can be done quite effectively at the Mn catalytic center. To act as catalytic scavengers of hydrogen peroxide, the oxomanganese salen complexes have to be deoxidized during part II of the reaction. Two possible reaction routes for the second part of the reaction: the top and the side substrate approach routes are discussed. Our findings could be the starting point for improvement of salen–manganese therapeutic and catalytic properties.

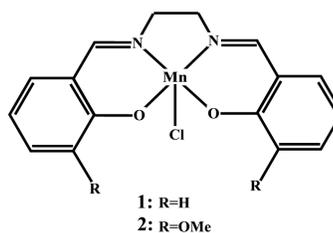
**Keywords:** catalase mimic, biomimetics, antioxidants, (salen)Mn, DFT

## INTRODUCTION

Salen-manganese complexes are known as synthetic asymmetric catalysts that are important alternative to enzymes because they give high enantioselectivity and, in contrast to biological catalysts, can be used with a wide range of substrates [1,2]. These complexes that perform as catalytic scavengers of reactive oxygen species may also have broad clinical applicability. An incomplete list of possible applications of synthetic antioxidants include treatment of Alzheimer's disease [3], stroke [4], heart disease [5], aging [6,7], and cancer [8].

(Salen)Mn(III) biomimetic compounds (Scheme 1)– have been proven to be especially promising as therapeutics [6,9]. The distinctive feature of the salen-manganese complexes that makes their broad pharmacological efficacy possible is the ability to mimic both catalase and superoxide dismutase enzymatic functions [10]. Remarkably, different salen-manganese compounds possess the same superoxide dismutase activity but may significantly differ in the catalase activity. For example, it was shown [11] that

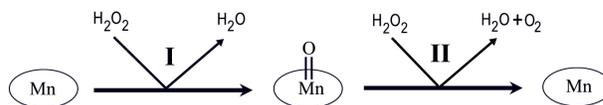
Scheme 1

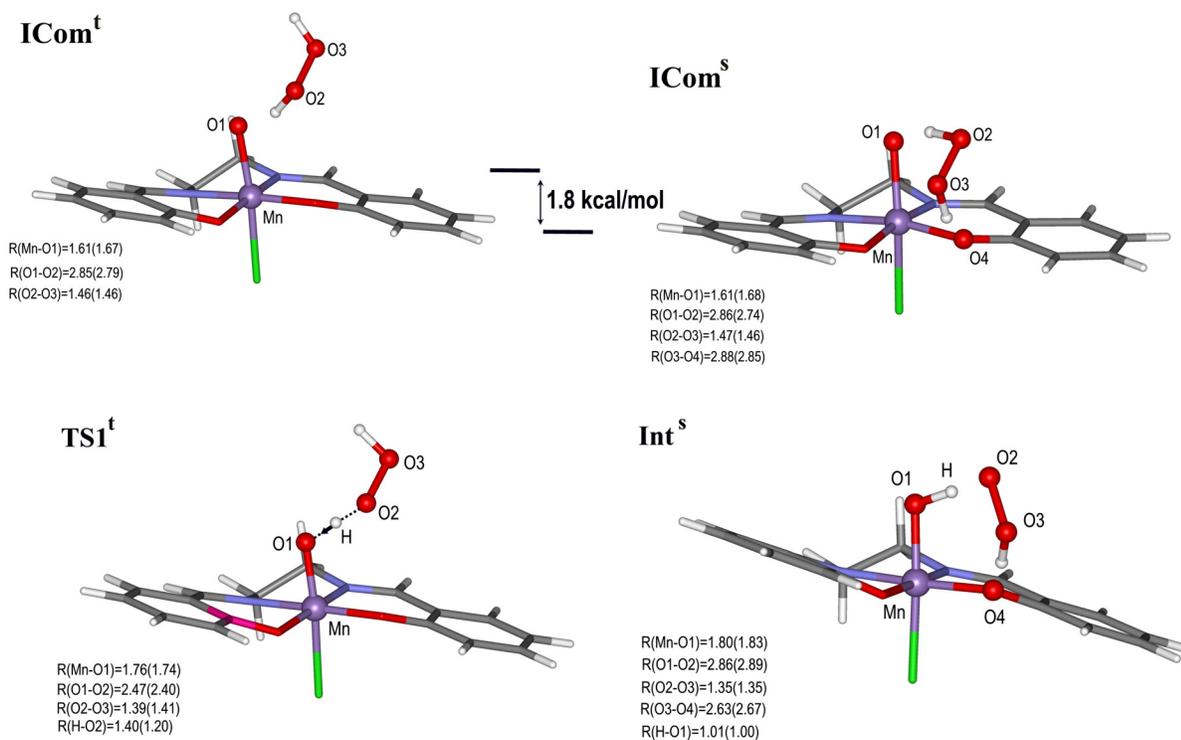


compound 2 is much more effective as a catalase than compound 1. In this paper, we present the results of theoretical investigation of salen-manganese complexes as catalytic scavengers of hydrogen peroxide molecules.

We consider (Scheme 2) a general “ping-pong” mechanism approach to describe the reaction process in which the first molecule of  $H_2O_2$  binds to the metal center (Mn(III)), oxidizes the metal and releases a molecule of water (I). The second peroxide molecule approaches the oxomanganese intermediate compound (Mn(V)) and the oxygen atom is transferred back to the peroxide molecule forming  $O_2$  and  $H_2O$  (II). The first part of the reaction was recently studied by us [12]. Our findings suggest that the first part of the dismutation reaction– the metal oxidation by a peroxide molecule–can be done effectively at the Mn catalytic center (the reaction TS barrier-3.6 kcal/mol). Here, we study step II of the dismutation reaction and, on the basis of our findings, suggest changes of salen-manganese compounds that should lead to better therapeutic properties. In addition, the basic mechanistic features for (salen)Mn compounds that perform as therapeutic agents or as synthetic asymmetric catalysts are discussed.

Scheme 2





**Figure 1.** Optimized critical reaction structures for the stepwise mechanism in the case of the top and the side approaches of the substrate. **ICom<sup>t</sup>** - initial complex corresponding to the top approach of the substrate; **ICom<sup>s</sup>** initial complex corresponding to the side approach of the substrate. The energetic difference between two initial complexes (1.8 kcal/mol) is also shown. **TS1<sup>t</sup>** - transition state (the top approach route) leading to the final product. **Int<sup>s</sup>** – intermediate on the side approach reaction route. Selected optimized geometrical parameters are shown for the singlet state. The corresponding parameters for the triplet state are shown in parentheses. Bond lengths are in angstroms.

## METHOD

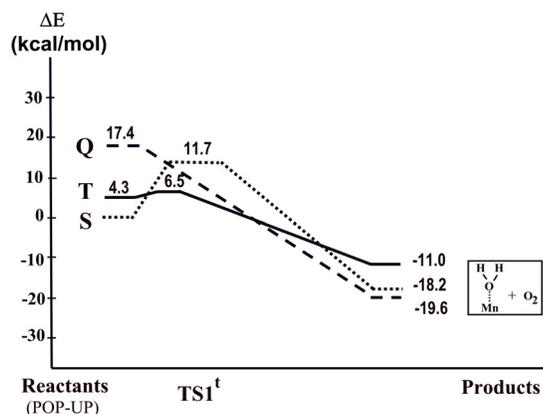
The real compounds **1** and **2** reacting with a peroxide molecule were utilized in our density functional theory (DFT) calculations to avoid uncertainties connected with using incomplete models. We use BP86 density functional that leads to conclusions that do not contradict to the qualitative picture of spin state pattern derived from high level ab initio calculations [13] of oxo-manganese species.

## RESULTS AND DISCUSSION

The reaction of converting H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> with simultaneous deoxygenation of the Mn atom of the oxomanganese salen **1** complex has been calculated on the three different spin potential energy surfaces: the singlet, the triplet and the quintet. The singlet state has been found to be the ground state for the initial reaction complexes followed by the triplet and the quintet states. Optimized structures for two possible conformations of the initial

reaction complexes are shown in Figure 1. The first structure **ICom<sup>t</sup>** corresponds to the top approach of a peroxide molecule to the plane of the catalase mimic. In this initial complex the substrate is situated in a “pop-up” position with respect to the catalyst plane. In contrast, the side approach of a peroxide molecule to the salen compound results in a stable complex **ICom<sup>s</sup>** that has a hydrogen bond not only between the O1 oxidizing atom of the catalyst and the O2 atom of the peroxide but also has the second hydrogen bond between the O3 atom of the peroxide and the O4 atom of the salen moiety. The energetic difference between these two initial complexes in the ground singlet state is just 1.8 kcal/mol with the complex **ICom<sup>s</sup>** being more stable than the complex **ICom<sup>t</sup>**. These two complexes are both accessible as starting conformations for this reaction. The mechanistic picture of the reaction strongly depends upon what initial complex the reaction originates from.

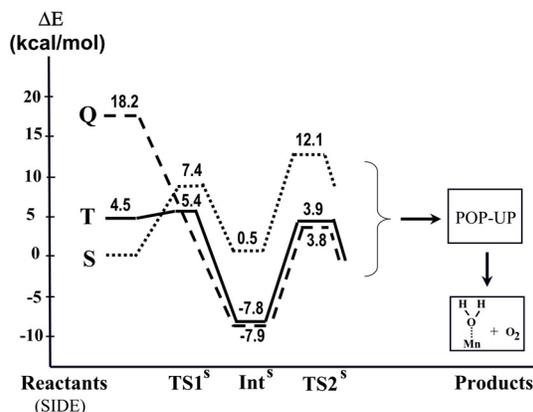
The energetic profiles for the reaction starting from the “pop-up” initial complexes on the singlet, triplet and quintet potential energy surfaces are shown in Figure 2. As can be seen from the Figure 2, the transfer of the protons from a



**Figure 2.** The reaction profiles in the case of the top approached substrate. Relative reaction energies of the triplet, the quintet and the singlet reaction pathways are shown.

peroxide molecule to the oxidizing oxygen atom of the oxomanganese salen compound can be done relatively easy. In the singlet state, which is the resting state of the reactants, a modest 11.7 kcal/mol activation energy is needed to overcome TS barrier for the transfer of the first proton from the substrate (Figure 1, Figure 2,  $\text{TS1}^t$ ). The triplet and quintet reaction channels are even more reactive: only a 2.2 kcal/mol barrier exists on the triplet path; there is no barrier in the quintet state. This reaction step can be considered as a spin-forbidden one and can be done very effectively by assuming a spin change during the reaction progress. In the case of this reaction, no intermediate structures have been found for the three spin channels. The reaction coordinate shows that after overcoming  $\text{TS1}^t$  barrier in the singlet and in the triplet states the intermediate reaction moiety H-O3-O2 (Figure 5) simply approaches the O1 axial atom of the Mn-salen compound and surrenders the second proton to the O1 atom without any barrier. Overall, the mechanism in the case of the top approach of the substrate is a one-step process.

The energetic profiles for the side approach reaction are shown in Figure 3. This reaction mechanism can be described as a number of steps starting from the initial reactant conformation  $\text{Icom}^s$  (Figure 1). First, the proton is transferred from the substrate to the O1 atom of the salen compound resulting in the reaction intermediate  $\text{Int}^s$ . For this intermediate structure the quintet and the triplet states are almost degenerated. They lie  $\sim 8$  kcal/mol lower than the singlet state, which is the resting state for the reactants. To finish the reaction, that is to transfer the second proton to the O1 atom of the catalyst, the hydrogen bond O3-H-O4 between the salen framework and the peroxide moiety has to be broken and the O2-O3-H peroxide part must be moved into the “pop-up” position with respect to the catalyst plane. Approximately the same barrier of



**Figure 3.** The reaction profiles in the case of the side approached substrate. Relative reaction energies of the triplet, the quintet and the singlet reaction pathways are shown.

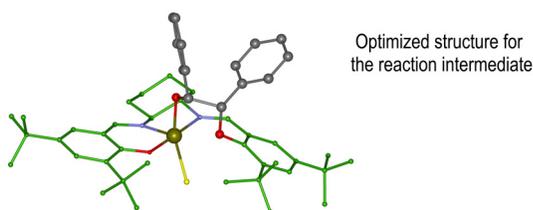
12 kcal/mol for every spin state has to be overcome to perform this reaction step. As soon as the O2-O3-H peroxide moiety is in the “pop-up” position, transfer of the second proton is a barrierless process in all three spin states as we discussed for the top approach reaction pathways.

The results of our calculations show that the mechanistic pictures for the top and the side approach reaction routes are quite different. The top approach reaction is a one-step process while the product formation in the case of the side approach of a peroxide molecule needs several steps. The principal difference of the side approach reaction route from the top one is the presence of a kinetically stable intermediate structure  $\text{Int}^s$  that appears on the reaction path after the first protonation of the O1 atom. The depth of the local minimum corresponding to this intermediate is  $\sim 12$  kcal/mol for the lowest triplet and the quintet states. The intermediate  $\text{Int}^s$  can be considered as an “energetic trap” on the reaction path, making the side approach route much less efficient than the one-step top approach transformation of a peroxide molecule. Taking into account that part I of the dismutation reaction (Scheme 2)—oxidation of the metal atom of the catalyst—can be done quite effectively [30] we conclude that the side approach reaction route for the catalyst recovery is the bottleneck for the whole dismutation reaction that follows a ping-pong mechanism. In other words, the catalyst could be at least temporarily deactivated (poisoned) if it entered the side approach reaction route due to formation of kinetically stable byproduct  $\text{Int}^s$ .

Based on this mechanistic picture one can speculate that blocking the side approach reaction route should lead to increasing the catalase activity of the (salen)Mn(III) compound. We suggest that bulky substituents at 3 and 3' positions of the salen ligand could block access of a peroxide molecule to the equatorial oxygen atoms of the salen framework. The latter should preclude formation of

the side approach initial reaction complex and “turn off” the side approach route. Indeed, the experimental evidence [11] suggests that substitution of the hydrogen atoms at 3 and 3' positions with R=O-CH<sub>3</sub> groups (Scheme 1, compound 2) dramatically improves the catalase activity of the salen biomimetic. Our preliminary modeling confirm that this substitution might shut down (at least, partly) the side approach reaction route. Thus, on the basis of the detailed knowledge of the mode of action of the (salen)Mn(III) catalase mimics, we suggest and rationalize structural changes of the catalyst that should lead to better therapeutic properties.

As we already mentioned, (salen)Mn complexes are also known as synthetic asymmetric catalysts that catalyze epoxidation of wide variety of unfunctionalized alkenes – the Jacobsen-Katsuki reaction. Despite extensive recent experimental and theoretical investigations, the Jacobsen-Katsuki epoxidation mechanism is still controversial. Quite recently we disclosed [14] an unprecedented (two-zone process with different spin-state channels) mechanistic picture for the Jacobsen-Katsuki reaction that could be used to explain a remarkable range of the experimental observations. The unique feature of the proposed mechanism is the formation of the five-member ring (FMR) intermediate with a covalent-like bond that is created between the carbon atom of the substrate and the oxygen atom of the salen framework (Figure 4).



**Figure 4.** Five-member ring reaction intermediate for the Jacobsen-Katsuki reaction

We stress that all previously proposed mechanisms (including radical one [13]), are based on the assumption that the oxidizing oxygen and Mn are the only atoms directly involved in the chemical reaction. The role of the salen frame is assumed to be “auxiliary”: to keep the proper oxidation state of the metal, to stabilize the catalyst as a chemical compound, and to govern the stereo interaction between the catalyst and the substrate. For the first time, we showed that the salen moiety of the catalyst can be explicitly involved in the epoxidation process (FMR reaction mechanism). One can easily see the analogy between mechanistic pictures of (salen)Mn catalase and catalyzed epoxidation activities: the presence of two competing reaction routes with different reaction characteristics. The top approach reaction route in peroxide dismutation process corresponds to the radical intermediate epoxidation pathway. Only the oxidizing oxygen –

substrate interactions are used to describe these reactions. The distinguish feature of the side substrate approach reaction route and the FMR epoxidation mechanism is the direct involvement of the salen framework in the chemical transformations. Our finding on the involvement of the salen ligand in the chemical reactions is a novel theoretically proven fact. We feel that such an innovative look at (salen)Mn chemical activity can be effectively used for rational tuning of the desired properties of (salen)Mn compounds. Overall, our findings on the mode of action of (salen)Mn compounds could be the starting point for structural and electronic tunings of the catalytic and therapeutic properties of these complexes.

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