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ARE NITRATE ESTERS LIKELY TO PRODUCE PEROXY CONTAINING SPECIES?

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Abstract. One of the most limiting factors in the use of organic nitrates as vasodilators in medicine is the development of nitrate tolerance. The chemistry leading to nitrate tolerance is not fully understood, newly formed reactive oxygen species, such as the peroxynitrite anion, have been suggested to play a central role. To elucidate the steps of the development of nitrate tolerance the aqueous phase interconversions of the isomeric methyl esters of peroxynitrous acid and nitric acid are studied computationally by quantum mechanical geometry optimizations and statistical mechanical methods. These calculations show that methyl nitrate is more stable than methyl peroxynitrite by about 134 kJ/mol. The energy of the transition state connecting the isomers is about 296 kJ/mol higher than that of methyl nitrate. This finding suggests that the direct isomerization of methyl nitrate to methyl peroxynitrite is of no importance at physiological conditions.

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Introduction

Different pharmacological molecules can stimulate vascular endothelial cells to synthesize and release vasorelaxing mediator endothelium derived relaxing factor (EDRF).[1] EDRF has been identified as nitric oxide synthesized by NO-synthase from L-arginine. After diffusion into vascular smooth muscle cells NO binds the heme moiety of soluble guanylyl cyclase and activates it. This event results in an elevation of intracellular cyclic guanosine monophosphate level with a subsequent relaxation of vascular smooth muscle.[2] Nitric oxide also inhibits platelet aggregation and adhesion[3] and reduces the extent of the leukocyte adhesion to the endothelial layer.[4] Abnormalities of the NO-releasing ability of vascular endothelial cells are relevant to pathophysiology of atherosclerosis,[5] hypertension[6] and coronary heart disease.[7]

It has been shown[8] that organic nitrates, widely used in medical therapy, are capable of relaxing vascular smooth muscle mimicking the effect of NO. One of the most limiting factors in the use of organic nitrates, especially the glycerol trinitrate (GTN), is the development of nitrate tolerance during the therapy. At one time the free thiol pool depletion was considered important, however, it is no longer considered to play a causal role.[9] In the light of recent findings[10-12], in *loco* newly formed peroxynitrite, $ONOO^-$, could play a central role in nitrate tolerance by generating serious oxidative stress and nitrating some aromatic sidechains in sensitive enzyme proteins. Beside the release of NO during GTN metabolism there is a considerable co-production of reactive oxygen species.[13] Among these molecules, the superoxide radical, O_2^-, may inactivate NO by producing peroxynitrite leading to impaired vascular relaxation and endothelial damage. The actual mechanism of NO release from GTN is not fully understood.[14] A recent mechanistic study suggests that the nitrite ion (NO_2^-) is released first, when GTN reacts with the free sulfhydryl groups of the enzyme mitochondrial aldehyde dehydrogenase.[15]

An alternative mechanism could involve an isomerization of the nitrate ester to a peroxy ester *via* a 1→2 (*i.e.*, N→O) alkoxy shift. In such an alternative mechanism the free -SH containing protein would enter at a later stage of mechanism. The first step of the mechanism is

$$R\text{-}O\text{-}NO_2 \rightarrow R\text{-}O\text{-}O\text{-}NO \qquad (1)$$

Peroxy esters are powerful oxidants, which make the proposed mechanism interesting in the context of recent studies of a connection between the nitrate tolerance and the level of oxidative stress.[10, 11, 16] Investigations of these reaction mechanisms also help us to estimate the potential beneficial effect of an antioxidant therapy to prevent or cure developing nitrate tolerance during nitrate therapy.

This paper examines the plausibility of the isomerization reaction, (1), on energetic grounds using quantum chemical optimization techniques. For practical reasons we have chosen to study a simple nitrate ester, *i. e.*, methylnitrate.

2. Method

The gas phase structures of methyl esters of peroxynitrous- and nitric acid are calculated by quantum chemical geometry optimizations using the software package Gaussian98.[17] The chosen level of theory is B3LYP, which is an implementation of the density functional method (DFT) method that has been used successfully in theoretical investigations of small molecules. We use two different basis sets, 6-31+G(d,p)[18] and AUG-cc-pVDZ[19], to probe the variation of the theoretical data with the basis set. The quality of the calculated thermodynamic data may be assessed by calculating the standard enthalpy change at 25°C for the esterification process

$$CH_3OH(g) + HNO_3(g) \rightarrow CH_3NO_3(g) + H_2O(g) \qquad (2)$$

We find $\Delta H°$ to be -31 and –23 kJ/mol at the levels B3LYP/6-31+G(d,p) and B3LYP/AUG-cc-pVDZ, respectively, while the experimental value derived from thermodynamic tables is -30 ± 1 kJ/mol. The difference between experimental and calculated bond lengths for the types of molecules considered here is of the order 1%. The determination of transition states (TS) is facilitated by using a synchronous transit-guided Quasi-Newton method.[20] Explicit calculations (IRC) were performed to identify the species linked by the transition states.

In the conventional therapy the organic nitrate is absorbed in body fluids with pH close to 7.2. We simulate this environment as pure water for simplicity. The thermodynamic properties of the species in aqueous solution are estimated using the approximate Tomasi theory of polarized continuum simulations, (PCM).[21, 22] That is, we find the energies of the various species in a medium with a dielectric constant of liquid water at 25°C. Normally this theory leads to good agreement with experiment for systems without

hydrogen bonding.[23] However, this shortcoming is likely to be of little importance here as we are considering differences between energies of similar molecules.

3. Results

3.1. *Methyl esters*

The optimized structures of the methyl esters of peroxynitrous- and nitric acid are depicted in Figs. 1-3. Methyl peroxynitrite appears in *cis* and *trans* (or *syn* and *anti*) forms (B and A in Figs. 1-3).

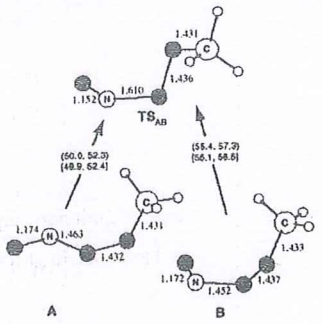

Figure 1. *Structures of cis-and trans-methyl peroxynitrite (B and A) and the transition state (TS_{AB}) that connects them. The structures are calculated at the B3LYP/6-31+G(d,p) level of theory. The distances and angles are in Å and degrees, respectively. ● indicates an oxygen atom and O indicates a hydrogen atom. The two numbers attached to the arrows refer to the energy separation (kJ/mol) of the involved structures. The first number represents the enthalpy difference at 0 K and the second number is the energy difference in a medium with the dielectric constant of liquid water at 25 °C. Numbers in parenthesis () and [] are derived at the B3LYP/6-31+G(d,p) and B3LYP/AUG-cc-pVDZ level of theory, respectively.*

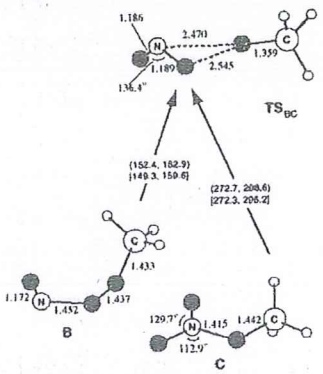

Figure 2. *Structures of cis-methyl peroxynitrite (B), methyl nitrate (C) and the transition state (TS_{BC}) that connects them. The structures are calculated at the B3LYP/6-31+G(d,p) level of theory. The symbols are explained in Fig. 1.*

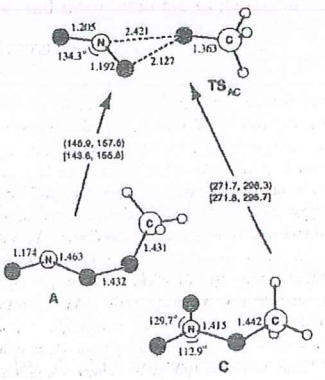

Figure 3. *Structures of trans-methyl peroxynitrite (A), methyl nitrate (C) and the transition state (TS_{AC}) that connects them. The structures are calculated at the B3LYP/6-31+G(d,p) level of theory. The symbols are explained in Fig. 1.*

In both conformers the CH_3O group is close to being perpendicular to the almost planar ONOO moiety. The optimized structure of methyl nitrate (C) is also included in Figs. 2 and 3. The energies of the *cis* and *trans* conformers (relative to methyl nitrate) are listed in Table 1. In this table and in the rest of the paper we list the result obtained with B3LYP/AUG-cc-pVDZ in parenthesis.

Conformer	ΔH°(0 K)	ΔE
Cis	120.4 (123.0)	133.7 (135.6)
Trans	125.9 (128.2)	138.7 (139.8)

Table 1. *Energy data for the cis- and trans isomers of the methyl ester of peroxynitrous acid relative to the methyl ester of nitric acid. The data are calculated at the B3LYP/6-31+G(d,p) and B3LYP/AUG-cc-pVDZ levels with the data for the latter given in parenthesis. ΔH°(0 K) is standard state enthalpy in the gas phase. ΔE is the energy in a continuum with the dielectric constant of liquid water at 25 °C. All energies are in unit of kJ/mol.*

It appears from Table 1 that the *cis* conformer is about 5 kJ/mol more stable than the *trans* conformer in the gas phase as well as in the aqueous phase. Methyl nitrate is more stable than the *cis*-peroxynitrous ester by 120.4 (123.0) and 133.7 (135.6) kJ/mol in the gas and aqueous phases, respectively.

3.2. *Transition states*

Three transition states have been identified. The transition state (TS_{AB}) that connects the *cis* and *trans* conformers of methyl peroxynitrite is included in Fig. 1. Structurally TS_{AB} may be considered as being derived from one of the peroxynitrous esters by an elongation of the ON-OO bond accompanied by a twist around that bond. The activation energy to this state is about 50-55 kJ/mol in the gas phase and a few kJ/mol higher in aqueous solution. The transition state TS_{BC} in Fig. 2 connects the *cis* conformer of methyl peroxynitrite with methyl nitrate. It appears from Fig. 2 that the activation energy to this state is substantially larger. In particular, the activation energy from methyl nitrate is 272.7 (272.3) kJ/mol in the gas phase and 296.6 (295.2) kJ/mol in aqueous solution. The two long bonds (dashed) in Fig. 2 make TS_{BC} look like a loose adduct of NO_2 and OCH_3.

The third transition state TS_{AC} in Fig. 3 connects the *trans* conformer of methyl peroxynitrite with methyl nitrate. TS_{AC} also looks like an adduct of NO_2 and OCH_3. A major structural difference between TS_{AC} and TS_{BC} is that all heavy atoms in the former are close to being coplanar. The energies of TS_{AC} and TS_{BC} are close, which implies that the activation energies from methyl nitrate to the *cis* and *trans* conformers are also close. Thus the activation energy from methylnitrate to both conformers of methylperoxynitrite is in excess of 295 kJ/mol in aqueous solution, which implies that the isomerization process is of no importance at physiological conditions.

In passing we note that loose transition states, similar to TS_{AC} and TS_{BC}, have also been identified in isomerizations of related species. Thus isomerization of the peroxynitrous acid, ONOOH, to nitric acid involves a loose NO_2-OH adduct[24] and the H_3CNO_2 rearrangement to $ONOCH_3$ involves a loose NO_2-CH_3 adduct.[25]

4. Discussion and Summary

The high activation energy for the isomerization of methyl nitrate to methyl peroxynitrite makes it unlikely that such drugs would be isomerized to dangerous peroxynitrite esters at body temperature without enzymatic catalysis. This finding also suggests that peroxynitrite generated during GTN metabolism most likely originates from the reaction of NO with the superoxide radical. It supports the hypothesis that radical trapping antioxidant therapy could help to avoid the development of nitrate tolerance by preventing the formation of protein nitrating peroxynitrite. The activation energy for the conversion of methylperoxynitrite into methylnitrate is found to be in excess of 156 kJ/mol in aqueous solution. This high value indicates that a direct conversion is unlikely and that a more elaborate mechanism is required to explain the findings of other researchers, that peroxynitrite can generate NO-releasing organic nitrates *in* vivo.[16, 26]

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