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# Semiempirical Molecular Orbital Calculation for the Redox Property of C-Terminal Active Site Sequence of Human Thioredoxin Reductase

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**Semiempirical molecular orbital calculation (MOPAC) was used to estimate the enthalpy difference (ΔH) between the reduced and oxidized states of the C-terminal redox center of human thioredoxin reductase. Heat of formation was computed by WinMOPAC 3.5Pro for the model peptides, *N*-Acetyl-Ser-Ile-Leu-Gln-Ala-Gly-X1-X2-Gly, whose-X1-X2-sequence was-Cys-SeCys-(natural sequence), -SeCys-Cys-(reverse sequence), -Cys-Cys, and-SeCys-SeCys-. Calculation by Hamiltonian AM1 and PM3 agreed that the oxidized state with selenosulfide bonds and a diselenide bond were more favorable than their reduced states. Only the peptide that contained-Cys-Cys-sequence was shown to have lower enthalpy when the two Cys were in the reduced form. It has been reported that substitution of SeCys498 to Cys results in the mutant TrxRs retaining only about 1% of the enzyme activity. The results of computational estimation supported the experimental hypothesis that the inactivation by SeCys498Cys mutation was due to the unfavorable formation of disulfide bond between Cys497-Cys498.**

**Key words :** MOPAC, thioredoxin reductase, selenocysteine, enthalpy

## Introduction

Thioredoxin reductase (TrxR) is a homodimeric flavoprotein that catalyzes the reduction of thioredoxin by NADPH. TrxRs characterized from a wide variety of species show significant amino acid sequence similarity, although they differ in size, structure, and catalytic mechanism. TrxRs from *Escherichia coli*[1] and *Saccharomyces cerevisiae*[2] are dimers of 35-kDa subunits, whereas TrxRs from higher eukaryotes, including mammals[3-5], *Caenorhabditis elegans*[6], and *Plasmodium falciparum*[7], are dimers of 55- to 58-kDa subunits. The major difference between the lower and higher molecular mass enzymes is that the latter contain an additional redox center preceding their C-terminal Gly; mammalian TrxRs have cysteine and selenocysteine (SeCys) residues in the conserved sequence (-Gly-Cys-SeCys-Gly), and *P. falciparum* TrxR has two Cys residues in the unique sequence (-Cys-Gly-Gly-Gly-Lys-Cys-Gly). Removal of the SeCys residue from mammalian TrxR by limited proteolysis[8], by oxidative selenium elimination, or by specific alkylation of the SeCys residue with bromoacetate at pH 6.5[9,10] led to a loss in enzyme activity, indicating an essential role for the SeCys. The Cys-SeCys pair was proposed to undergo redox change through selenosulfide bridge formation during its turnover. It has been reported that the replacement of SeCys with Cys results in a mutant TrxR enzymes with about 1% activity with thioredoxin as substrate, mostly due to the loss in *kcat*, indicating absolute requirement of selenium for the catalysis[11].

The present study has been undertaken to investigate the theoretical background for these experimental studies. Semi-empirical molecular orbital calculation, MOPAC[12], was employed to estimate the energy levels of oxidized and reduced forms of the model peptide, *N*-Ac-Ser-Ile-Leu-Gln-Ala-Gly-X1-X2-Gly, in which X1 and X2 were either Cys or SeCys. The calculation was carried out for gas phase and for aqueous phase; three Hamiltonians, AM1[13], PM3[14-15], and PM5, which was released in 2001, were tested. Hamiltonian AM1 and PM3 gave comparable results for both gas phase and aqueous phase calculation, oxidation of two adjacent Cys residues was found to be unfavorable compared to the oxidation of SeCys-containing sequences. In contrast, PM5 failed to give a rationale for the substitution effects of sulfur for selenium, and it was considered not appropriate for this investigation.

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## Methods

A personal computer LaVie LC500/2 (NEC, Japan), implemented with Pentium III 900 MHz CPU and 384 MB memory, was installed with the operating system Windows XP Home edition (Microsoft, USA). CS Chem3D Pro (CombridgeSoft Corporation, USA) was used to construct the initial structure of model peptides. Four sequences were made by typing letters as AcSerIleLeuGlnAlaGlyCysCysGlyOH. The oxidized peptides were made by erasing hydrogen atoms from the sulfur atoms and by connecting the two sulfurs by a single bond. Sulfur atom was replaced with selenium when the sequence contained SeCys residue. The structures were optimized by molecular mechanistic optimization MM2 and saved as the MOPAC INPUT format (*.MOP). Mop files were opened on WinMOPAC 3.5 Pro (FUJITSU, Japan), and the structure was further optimized by the molecular orbital calculation. Reduced and oxidized forms of peptides were calculated in gas phase and in aqueous phase[16], and AM1, PM3, and PM5 were chosen for the Hamiltonian. Taking three Hamiltonians and two conditions (gas phase or aqueous phase) gave 6 results for each pair of reduced and oxidized states of the four peptide sequences. The heat of formation for hydrogen molecule was summed up to the oxidized peptide, and the enthalpy difference between oxidized ($Hox+H_{H2}$) and reduced (Hred) forms were represented by the subtraction, $\Delta H = Hox + H_{H2} - Hred$.

## Results and Discussion

Both the Hamiltonian AM1 and PM3 gave comparable results for gas phase and for aqueous phase calculations (Table 1). Two model peptides that form selenosulfide bond and the one that forms diselenide bond gave negative ΔH values. These sequences appeared to be more stable in the oxidized form than in the reduced form. Diselenide bond formation had an effect of giving the lowest ΔH values among others. Two sequences of selenosulfide bonds, in natural and reverse orders, showed negative ΔH values which were close to each other, suggesting that the position was not a significant factor for ΔH. In contrast, the sequence that has adjacent two Cys residues gave positive ΔH value, suggesting that the sequence was more stable in the reduced form than the oxidized form. Calculation for gas phase and aqueous phase gave similar results by AM1 and PM3. The solvation effect was smaller in AM1 calculation (less than 5 kcal/mol) than in PM3 (less than 9 kcal/mol) but their difference was not very significant. Since the Hamiltonian choice and solvation effects did not alter the relative ΔH values of the four sequences, these results may represent the essential chemical properties of these redox-active sequences.

The PM5 Hamiltonian appeared to have some computational problems in calculating these peptides; the problem appeared more evident when the calculation involved solvation effects. In gas phase, the peptide that has-Cys-Cys-sequence gave the largest ΔH decrease (−28.3 kcal/mol), suggesting that disulfide bond formation stabilizes the molecule more effectively than the formation of diselenide and selenosulfide bonds. In contrast, aqueous phase calculation gave positive ΔH values for all the four peptides, suggesting that the redox center prefers to be in the reduced form. The largest ΔH increase (16.6 kcal/mol) by the -Cys-Cys- sequence now suggests that reduction may stabilize the molecule. Thus, PM5 computation suggests that the enthalpy change (ΔH) may not be affected by the chemistry of sulfur and selenium, but is rather affected by solvation effects, which is computed by the function of dielectric constant of the clusters of solvent molecules[16].

The effect of replacing SeCys498 with Cys has been experimentally demonstrated by using a mutant form of human placental TrxR1 expressed in *Escherichia*

Table 1 Redox-dependent enthalpy change (ΔH) of model peptides

| Sequence | ΔH ($Hox+H_{H2}-Hred$) kcal/mol | | |
|---|---|---|---|
| | AM1 | PM3 | PM5 |
| Ac-SILQAG-Cys-SeCys-G | −13.3092 (gas) | −25.1374 (gas) | −5.66252 (gas) |
| (normal sequence) | −18.3109 (aqueous) | −16.0092 (aqueous) | 8.44211 (aqueous) |
| Ac-SILQAG-SeCys-Cys-G | −15.8507 (gas) | −21.3062 (gas) | −5.73718 (gas) |
| (reverse sequence) | −19.7041 (aqueous) | −22.3837 (aqueous) | 9.94794 (aqueous) |
| Ac-SILQAG-Cys-Cys-G | 6.34379 (gas) | 1.12062 (gas) | −28.3224 (gas) |
| (dithiol/disulfide) | 6.03412 (aqueous) | 7.71041 (aqueous) | 16.64166 (aqueous) |
| Ac-SILQAG-SeCys-SeCys-G | −36.3816 (gas) | −35.4871 (gas) | −14.6295 (gas) |
| (diselenol/diselenide) | −34.5301 (aqueous) | −43.5802 (aqueous) | 5.59611 (aqueous) |

*coli*[17]. The NADPH-disulfide oxidoreductase activity of the Cys498 mutant enzyme was 6% of wild-type TrxR1 when 5, 5'-dithio-*bis* (2-nitrobenzoic acid) was used as a substrate. Disulfide bond formation between Cys497-Cys498 residues by excess $TrxS_2$ was 12% of that of the wild type that has Cys497-SeCys498. Accordingly, biochemical experiments have led to the hypothesis that formation of a disulfide bond between two adjacent Cys residues may be unfavorable because of the distance between sulfur atoms, and also because it requires constraining the intra-cysteinyl peptide bond to the *cis* rather than the preferred *trans* orientation[18]. The larger atomic size of selenium is thought to obviate these unfavorable characteristics. The semi-empirical molecular orbital calculation using AM1 and PM3 Hamiltonian has supported the biochemical studies by estimating the enthalpy change ($\Delta H$) by the redox states of the redox-active C-terminal sequences.

The present calculation was limited to the peptide molecule modeled after the sequence from Ser491 to Gly499 in human TrxR1, whose subunit is composed of 499 amino acids. Although the enthalpy difference between the natural (-Cys-SeCys) and reversed (-SeCys-Cys) sequences was similar, the order of Cys and SeCys may be more important when the C-terminal redox center is involved in the enzyme reaction. It would be interesting if the mutant enzymes with SeCys-Cys (reverse sequence), and SeCys-SeCys (diselenol form) sequences at the C-terminal end were prepared as the recombinant TrxR in *E. coli*.

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# ヒト由来チオレドキシン還元酵素のカルボキシル末端配列の酸化還元状態の半経験的分子軌道計算

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ヒトのチオレドキシン還元酵素（TrxR）のカルボキシル末端配列の酸化還元に伴うエンタルピー変化を計算した．半経験的分子軌道計算 WinMOPAC 3.5Pro を用いてモデルペプチド，*N*-Ac-Ser-Ile-Leu-Gln-Ala-Gly-X 1-X 2-Gly の生成熱を算出した．X 1，X 2 のアミノ酸配列は-SeCys-Cys-，-Cys-SeCys-，-SeCys-SeCys-，-Cys-Cys-のいずれかで，それぞれ酸化状態と還元状態を計算し，そのエンタルピー差を求めた．ハミルトニアン AM 1 と PM 3 は同じ傾向の計算結果を示し，セレノスルフィドまたはジセレニドを形成するペプチドは酸化状態でより安定化するのに対して-Cys-Cys-の配列を持つペプチドは還元型の方が安定であることを示した．ほ乳類の TrxR の SeCys498 を Cys に置換すると酵素活性が１％程度にまで低下することが報告されている．これは，変異酵素が-Cys497-Cys498-間で酸化的架橋を形成しにくいからと考察されていたが，今回の分子軌道計算の結果は，この仮説を支持している．