

Examination of the Reduced Affinity of the Thymidylate Synthase G52S Mutation for FdUMP by Ab Initio and Semi-empirical Studies

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Abstract

Background: The G52S mutation in the Arg⁵⁰ loop of thymidylate synthase leads to decreased binding of FdUMP. It has been suggested that the mutation affects the Arg⁵⁰ residue (within the Arg⁵⁰ loop) responsible for binding the phosphate of FdUMP. The binding of the methylguanidinium moiety as a model for Arg⁵⁰ to a methylphosphate entity as a model for FdUMP was investigated with theoretical calculations, as well as the structure of the Arg⁵⁰-Thr⁵¹-Gly⁵² tripeptide in comparison with the Arg⁵⁰-Thr⁵¹-Ser⁵² tripeptide.

Methods: Gaussian-98 and PC Spartan programs were used to perform Hartree-Fock and Post-Hartree-Fock

quantum chemical calculations as well as MNDO (semi-empirical calculations).

Results: It was found that the strongest binding occurs between the negative methylphosphate ion and methylguanidine. The replacement of Gly⁵² by Ser⁵² leads to a significant displacement of Arg⁵⁰, which may be responsible for the decreased binding to FdUMP.

Conclusion: The arginine-phosphate binding appears to be geometry dependent. Thus, the displacement of the Arg⁵⁰ residue, as observed in these calculated models, upon mutation of Gly⁵² to Ser may contribute to decreased binding of FdUMP to mTS (G52S).

Introduction

Thymidylate synthase (TS) is an essential enzyme in proliferating cells and an important target for a variety of anticancer drugs. This enzyme is responsible for catalyzing the de novo biosynthesis of thymidylate, which is necessary for DNA synthesis and repair. The mechanism involves the reductive transfer of a methylene group from the cofactor, 5, 10-methylene-tetrahydrofolate (CH₂H₄ folate), to the 5 position of the substrate, 2'-deoxyuridine 5'-monophosphate (dUMP), to form 2'-deoxythymidine 5'-monophosphate (dTMP).

Many TS analogs of both the substrate, dUMP, and the cofactor, CH₂H₄folate, have been synthesized and investigated as potential anticancer therapeutics. Two clinically useful substrate analogs are 5-fluorouracil (5-FU) and fluorodeoxyuridine (FdUrd), which are metabolized in vivo to 5-fluoro-2-deoxyuridylate (FdUMP). FdUMP acts as an inhibitor of TS by forming a stable ternary complex with TS and the folate cofactor, resulting in inhibition of enzyme function. In addition to these sub-

strate analogs, novel folate cofactor analogs, such as raltitrexed (Tomudex) and thymitaq have also been designed as specific inhibitors of TS activity (1a).

To better understand the mechanism of TS enzyme catalysis, several laboratories have correlated TS enzyme structure and activity using mutagenesis. Following substitution of different amino acids at random or specific points within the enzyme, enzyme activity and affinity for inhibitors are measured to determine how a particular residue(s) contributes to the overall mechanism. Recently, several mutations at highly conserved active site residues have been made for human TS (hTS) (Ile¹⁰⁸, Leu²²¹, and Phe²²⁵)(1b-e). The I108A mutant confers resistance to raltitrexed and thymitaq with respective IC₅₀ values of 54 and 80 times greater than the wild-type enzyme. These experiments led Sapse et al. (2) to perform quantum chemical ab initio calculations on the complexes formed by the binding of thymitaq with various mutants of Ile¹⁰⁸.

In this study, a mutant, G52S, within the Arg⁵⁰ loop of TS was examined. This mutant, obtained using EMS mutagenesis, was found to retain full catalytic activity with a reduced affinity for thymitaq and FdUMP having Ki values of 5 and 20 times greater than that of wt TS, respectively. Other mutations in

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this region, such as R50C, T51A, and D49N, were shown to greatly affect the activity of the enzyme. The reduced affinity of the G52S mutant for thymitaq and FdUMP was postulated to result from impaired movement of the Arg⁵⁰ loop due to the effect of the G52S mutation, thereby compromising the interaction of the loop with nucleotide and folate molecules (3).

Upon binding of the substrate (dUMP) to the active site of TS, Arg⁵⁰ and Gly⁵² side-chains reorient to accommodate binding to the phosphate moiety of dUMP. This reorientation results in a slight shift (<1 Å) of the entire Arg⁵⁰ loop, a step necessary to accept the incoming folate molecule. Once the ternary complex is formed a substantial conformational change in the enzyme's tertiary structure occurs. The carboxylate of the C-terminal valine moves into the active site where it and N-1 of CH₂H₄folate form hydrogen-bond networks with Arg⁵⁰ through fixed H₂O molecules. Thus, this flexible Arg⁵⁰ residue acts as a bridge linking the enzyme C terminus, substrate, and cofactor together and plays an important role in the enzyme's mechanism of action by binding to the phosphate group of dUMP (4).

To examine the hypothesis that the reduced affinity of G52S for Thymitaq and FdUMP is a result of impaired movement of the Arg⁵⁰ loop and to investigate further the Arg-FdUMP interaction, this study applied *ab initio* (Hartree-Fock) and semi-empirical calculations to the system.

Methods and Results

Interactions Between the Methylguanidinium Ion as a Model for the Arg⁵⁰ Residue and the Phosphate Group as a Model for FdUMP in TS

The Gaussian 98 computer program (5) was used to perform *ab initio* calculations at HF/6-311G* (Hartree-Fock) and at MP2/6-311G* level to investigate the interaction between the methylguanidinium ion as a model for the Arg⁵⁰ residue in thymidylate synthase, and a phosphate group as a model for the inhibitor FdUMP. The 6-311G* basis set involves one Slater orbital for the description of core electrons and three Slater orbitals for the description of valence electrons, one expanded in a series of three gaussians and the other two approximated by one gaussian each. In addition, d functions are set on the non-hydrogen atoms. The correlation energy is included in the calculations via the use of the Moller-Plesset perturbation theory, with the second-order term, MP2, applied to all electrons. The two systems considered are

- Complex 1a—the complex formed by methylguanidine and the negative methyl phosphate ion (Fig. 1A), and
- Complex 1b—the complex formed by the methylguanidinium ion with the binegatively charged methyl phosphate ion (Fig. 1B).

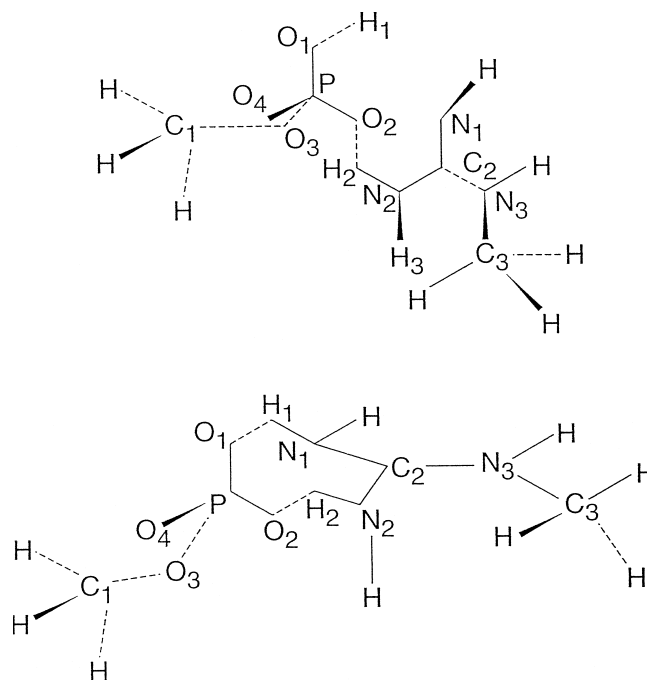


Fig. 1. (A) The complex formed by the methylguanidine and the negative methyl phosphate ion. (B) The complex formed by the methylguanidinium ion and the binegative methyl phosphate ion.

The energies of the complexes and of the subsystems, as obtained via geometry optimization, are shown in Table 1. Table 2 shows the optimized parameters of the complexes.

Comparison of the Structures of the Arg⁵⁰-Thr⁵¹-Gly⁵² and the Arg⁵⁰-Thr⁵¹-Ser⁵² Peptides

The blocked Arg⁵⁰-Thr⁵¹-Gly⁵² and Arg⁵⁰-Th⁵¹-Ser⁵² tripeptides are geometry optimized using the 6-31G* basis set at Hartree-Fock level, as implemented by the PC Spartan program (6).

Figure 2A shows the optimized Arg⁵⁰-Thr⁵¹-Gly⁵² tripeptide and Figure 2B shows the Arg⁵⁰-Thr⁵¹-Ser⁵² optimized tripeptide. Figure 2C shows the two peptides superimposed at the threonine residue.

The Arg⁵⁰ loop in hTS (Arg⁴⁶-Lys⁴⁷-Asp⁴⁸-Asp⁴⁹-Arg⁵⁰-Thr⁵¹-Gly⁵²-Thr⁵³-Gly⁵⁴-Thr⁵⁵) was op-

Table 1. Energies of the complexes and subsystems at MP2 (full)/6-311G* computational level (au)

| | |
|----------------------------------|------------|
| Complex 1a | -925.96762 |
| Complex 1b | -925.91718 |
| Methylphosphate (negative ion) | -681.86106 |
| Methylphosphate (binegative ion) | -681.11432 |
| Methylguanidine | -244.07450 |
| Methylguanidinium ion | -244.47037 |

Table 2. Geometric parameters of the complexes (Å and degrees) optimized at MP2 (full)/6-311G* level

| Group | Bond Lengths | |
|-------|--------------|------------|
| | Complex 1a | Complex 1b |
| PO1 | 1.654 | 1.552 |
| PO2 | 1.503 | 1.542 |
| PO3 | 1.657 | 1.698 |
| PO4 | 1.487 | 1.506 |
| C1O3 | 1.414 | 1.403 |
| H1O1 | 0.960 | 1.510 |
| N1H1 | 4.040 | 1.01* |
| C2N1 | 1.283 | 1.322 |
| C2N2 | 1.394 | 1.318 |
| C2N3 | 1.406 | 1.370 |
| C3N3 | 1.453 | 1.443 |
| O2H2 | 1.875 | |
| O3H3 | 2.300 | |

| | Bond Angles | |
|--------|-------------|------------|
| | Complex 1a | Complex 1b |
| O1PO2 | 107.9 | 110.6 |
| O2PO3 | 100.1 | 103.4 |
| O3PO4 | 107.3 | 115.3 |
| C1O3P | 116.0 | 113.8 |
| H1O1P | 109.0 | 122.2 |
| N1H1O1 | 236.0 | 180.0* |
| C2N1H1 | 122.8 | 122.0 |
| N2C2N1 | 127.9 | 120.7 |
| N3C2N2 | 111.6 | 119.9 |
| C3N3C2 | 115.7 | 123.4 |

| | Dihedral Angles | |
|----------|-----------------|------------|
| | Complex 1a | Complex 1b |
| O3PO1O2 | 108.0 | 107.2 |
| O4PO1O2 | -137.9 | -136.2 |
| C1O3PO2 | 186.6 | 177.3 |
| N1H1O1O2 | 0.0 | 0.0 |

*Kept frozen.

timized with the MNDO semi-empirical method and modeled using the Insight II program on a Silicon Graphics station. The same procedure was used for the loop with Gly⁵² replaced by a Ser residue. The superimposed peptides of the Arg⁵⁰ loop are shown in Figure 3. Figure 4 shows the superimposition of

the tripeptides Arg⁵⁰-Thr⁵¹-Gly⁵² and Arg⁵⁰-Thr⁵¹-Ser⁵² as optimized by the MNDO method and modeled by Insight II. Figure 5 shows the loop as part of rat thymidylate synthase complexed with dUMP and the anticancer drug raltitrexed (Tomudex) (7).

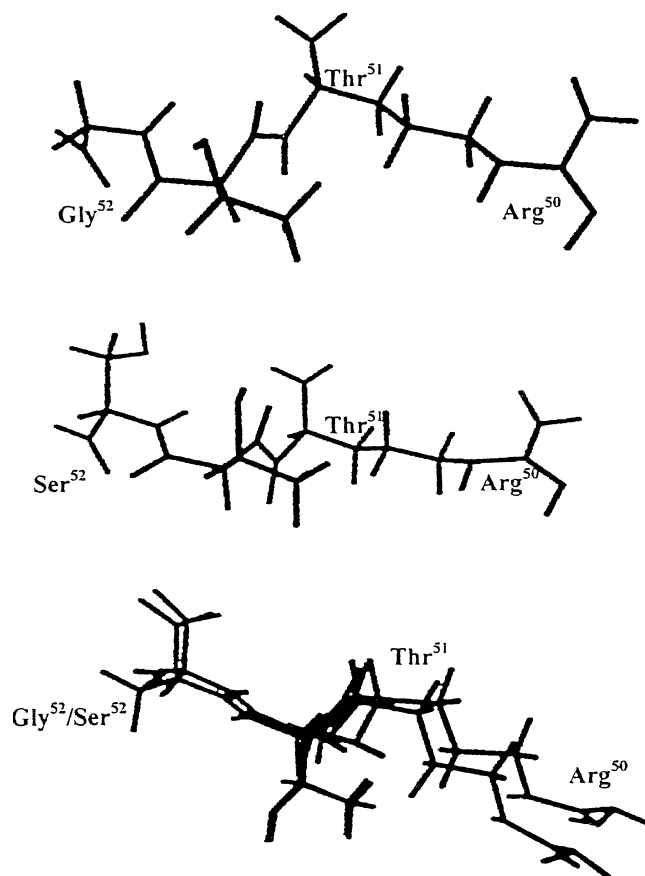


Fig. 2. Optimization of the tripeptide at HF/6-31G* level. (A) Arg⁵⁰-Thr⁵¹-Gly⁵² optimized at HF/6-31G* level. (B) Arg⁵⁰-Thr⁵¹-Ser⁵² optimized at HF/6-31G* level. (C) Arg⁵⁰-Thr⁵¹-Gly⁵² and Arg⁵⁰-Thr⁵¹-Ser⁵² superimposed at Thr⁵¹ residue.

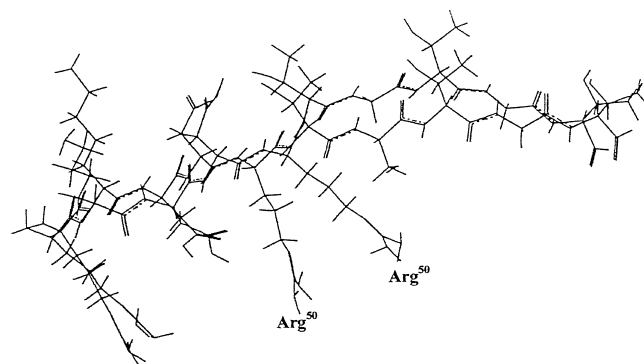


Fig. 3. The wild-type Arg⁵⁰ loop and the Arg⁵⁰ loop with the G52S mutation optimized at MNDO level and superimposed.

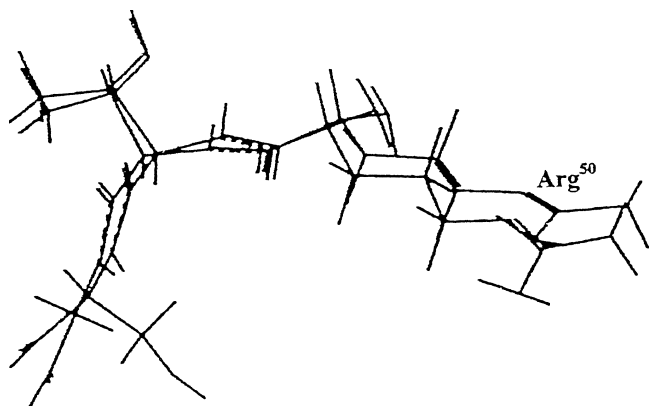


Fig. 4. Arg⁵⁰-Thr⁵¹-Gly⁵² and Arg⁵⁰-Thr⁵¹-Ser⁵² optimized at MNDO level and superimposed.

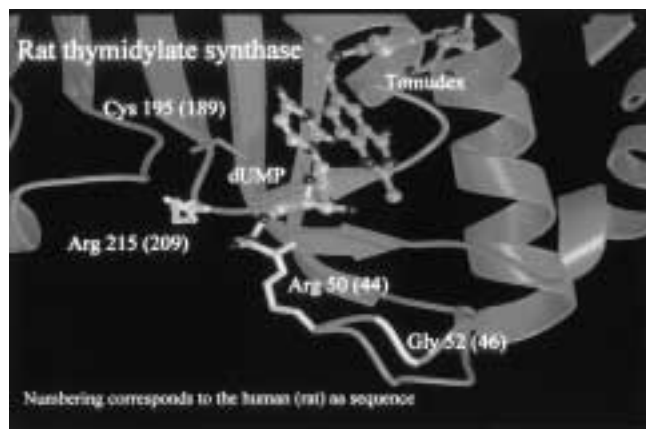


Fig. 5. The Arg⁵⁰ loop as part of rat thymidylate synthase (9).

Discussion

The complex formed by the negative methylphosphate and methylmethylguanidine (Complex 1a; Fig. 1A) is more stable than the one formed by the methylmethylguanidinium ion and the binegative methylphosphate ion (Complex 1b; Fig. 1B), by 31.80 kcal/mol at HF/6-311G*//MP2 (full)/6-311G* level and by 31.64 kcal/mol at MP2 (full)/6-311G* level. This is not surprising: the proton affinity of methylguanidine is only 257.38 kcal/mol at HF/6-311G*//MP2 (full)/6-311G* level and 248.41 kcal/mol at MP2 (full)/6-311G* level, whereas the proton affinity of the binegative methylphosphate ion is 473.29 kcal/mol at HF/6-311G*//MP2 (full)/6-311G* level and 468.58 kcal/mol at MP2 (full)/6-311G* level. On the other hand, complex 1b features a much larger binding energy due to the electrostatic attraction between opposite charges, in addition to the hydrogen binding. Indeed, the binding of complex 1a is 16.40 kcal/mol at HF/6-311G*//MP2 (full)/6-311G* level and 20.12 kcal/mol at MP2 (full)/6-311G* level, while the binding energies of

complex 1b are 200.51 kcal/mol and 208.64 kcal/mol, respectively; these energies include an electrostatic interaction between a positive and a negative charge.

Complex 1a was optimized by allowing the methylguanidine moiety to relax into a nonplanar conformation, similar to methylguanidine itself. Indeed, if methylguanidine is optimized using a small basis set, without energy terms, it adopts a quasi-planar geometry. However, when large basis sets such as 6-311G* are used, methylguanidine features the hydrogens out of the plane of the carbon and the three nitrogens. On the contrary, the guanidinium ion is always planar, due to the Y aromaticity. As a consequence, when the methylguanidine moiety is forced to stay planar, the most stable complex features a hydrogen bond between H1 (placed on the oxygen O1) and the nitrogen atom N1, on which there is only one hydrogen. This complex has a binding energy about half of the complex 1a, which features one of the hydrogens set on nitrogen N2 hydrogen bound to O2, at a distance of 1.88 Å. It may be concluded that the strongest phosphate-methylguanidine interaction occurs via the hydrogen bond formed by one of the hydrogens set on a nitrogen and an oxygen of the phosphate ion. As shown before, this complex is more stable than complex 1b.

As seen from Figure 2C, the HF/6-311G* optimization of the Arg⁵⁰-Thr⁵¹-Gly⁵² and the Arg⁵⁰-Thr⁵¹-Ser⁵² predicts a significant displacement of the Arg⁵⁰ residue when Gly⁵² is replaced by Ser. The difference in the geometries of the two tripeptides might be attributed to electrostatic effects introduced by the presence of the electronegative oxygen present in serine.

When the whole Arg⁵⁰ loop is optimized with the MNDO method, as seen in Figure 3, the displacement of Arg⁵⁰ is seen even more. This displacement is also observed in Figure 4, which shows the MNDO optimization of the two tripeptides.

In conclusion, our calculations of the interaction between the methylguanidinium ion as a model for the Arg⁵⁰ residue and the phosphate group as a model for FdUMP in TS predict a significant displacement of the Arg⁵⁰ residue upon mutation of Gly⁵² to Ser. It is possible that this displacement could result in impaired movement of the entire Arg⁵⁰ loop, accounting for loss of binding of FdUMP.

Because loss of binding is not seen with the natural substrate, dUMP, it is suggested that the presence of the fluorine on FdUMP may influence the flexibility of the phosphate group.

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