Bioorganic Chemistry **28,** 156–162 (2000)
doi:10.1006/bioo.2000.1172, available online at http://www.idealibrary.com on IDEAL®

Different Activities of 5-Hydroxy-dUMP and 5-Hydroxymethyl-dUMP in Thymidylate Synthase-Catalyzed Reaction in View of Molecular Modeling and Structural Studies

Adam Jarmuła,* Andrzej Leś,† and Wojciech Rode*

**Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw; and †Department of Chemistry, University of Warsaw, 1 Pasteur Street, 02-093 Warsaw, Poland*

Received January 28, 2000

In order to explain different activities shown by 5-hydroxy-dUMP (substrate) and its close analogue 5-hydroxymethyl-dUMP (slow-binding inhibitor) in the reaction catalyzed by thymidylate synthase, studies have been undertaken involving (i) *ab initio* RHF simulations, (ii) comparative analysis of crystallographic structures available from CSD, and (iii) QSAR analysis of experimental results describing thymidylate synthase interaction with various 5-substituted dUMP analogues. Assuming substrate activity of 5-hydroxy-dUMP to be associated with proton release from the C(5) hydroxyl in the enzyme-catalyzed reaction, acidities of 5-hydroxy and 5-hydroxymethyl substituents in dUMP molecule were compared. The results indicate the 5-hydroxyl deprotonation to be easier and supported by resonance electronic effect, pointing to a probable mechanism of different activities of the two dUMP analogues in thymidylate synthase reaction. The possibility is discussed that 5-mercapto-dUMP and 5-hydroseleno-dUMP, previously assumed to be inhibitors, could be also substrates for thymidylate synthase, as the 5-mercaptyl and 5-hydroselenidyl appear to be deprotonated even more easily than the 5-hydroxyl.

Key Words: 5-hydroxy-dUMP; 5-hydroxymethyl-dUMP; 5-mercapto-dUMP; thymidylate synthase; *ab initio* RHF; QSAR.

INTRODUCTION

Thymidylate synthase (EC 2.1.1.45), a target enzyme in chemotherapy, catalyzes $N^{5,10}$-methylenetetrahydrofolate (CH_2H_4PteGlu)-dependent methylation of dUMP C(5). Thymidylate and dihydrofolate are the reaction products (*1*). Our recently published results indicated a new substrate analogue, 5-hydroxy-dUMP (oh^{5}dUMP), to be a substrate, as reflected by $N^{5,10}$-methylenetetrahydrofolate consumption indicated by results of spectrophotometric monitoring of the reaction (*2*). It was very slowly (some 20-fold slower than dUMP) processed but well recognized by the enzyme, with a K_i^{app} value, reflecting oh^{5}dUMP competition with [5-^{3}H]dUMP, in the range of the K_m value for dUMP. In contrast, a product analogue, 5-hydroxymethyl-dUMP (hm^{5}dUMP), behaved as a slow-binding inhibitor, exhibiting, in the presence

0045-2068/99 $30.00

of CH_2H_4PteGlu, time-dependent inactivation of the enzyme (*2*). In an attempt to appreciate the mechanism of different influences of the C(5)–OH and C(5)–CH_2OH substituents on the interaction with thymidylate synthase, we directed our attention to the C(5) proton release from the pyrimidine moiety, one of the key steps in a thymidylate synthase-catalyzed reaction (*3*). Assuming the reaction with oh^{5}dUMP to involve proton release from the C(5) hydroxyl, molecular modeling RHF studies, analysis of available structural data, and QSAR studies were undertaken, aimed at comparison of acidities of 5-hydroxy and 5-hydroxymethyl substituents. Besides considering a possibility that previously described 5-mercapto-dUMP (*4*) and 5-hydroseleno-dUMP (*5*) could be enzyme substrates, the molecular modeling RHF studies have been extended in order to assess deprotonation of the 5-mercaptyl and 5-hydroselenidyl substituents.

RESULTS

Ab Initio RHF

The *ab initio* RHF/6-31G** calculations were performed for the neutral and anionic (deprotonated) forms of 1-methyl-5-hydroxy (-mercapto; -hydroseleno)-5-aminomethyl-6-thiomethyl-5,6-dihydro-uracil (H, SH, or SeH, respectively) and 1-methyl-5-hydroxymethyl-5-aminomethyl-6-thiomethyl-5,6-dihydrouracil (HM) models of 5-hydroxy-dUMP (5-mercapto-dUMP; 5-hydroseleno-dUMP) and 5-hydroxymethyl-dUMP in ternary complexes with thymidylate synthase and CH_2H_4PteGlu (Fig. 1). For comparison, neutral and anionic forms of methanol, phenol, and benzyl alcohol were also considered. The total molecular energy differences (calculated for the molecular geometries optimized for all geometrical parameters) between anionic and neutral forms of H (−374.5 kcal/mol) and HM (−383.6 kcal/mol) showed the hypothetical deprotonation of the C(5) hydroxyl in H to be reminiscent of that of phenol (−373.9 kcal/mol) and to be easier than deprotonation of HM (−383.6 kcal/mol) or still less acidic, benzyl alcohol (−396.9 kcal/mol) and methanol (−412.4 kcal/mol) (Table 1). On the other hand deprotonation of both the C(5) mercaptyl in SH (−348.9 kcal/mol) and the C(5) hydroselenidyl in SeH (−347.8 kcal/mol) was much easier than deprotonation of H (Table 1).

Structural Data from CSD

The comparative analysis was made of crystallographic structures (available from Cambridge Structural Database (CSD), all solved at high precision, as indicated by AS1 flag (*6*)), of 5-substituted analogues of uridine and 2′-deoxyuridine, including 5-hydroxyuridine (*7*), 5-methoxyuridine (*8*), 5-hydroxymethyl-2′-deoxyuridine (*9*), and 5-methoxymethyl-2′-deoxyuridine (*10*), modeling the corresponding analogues of dUMP. In 5-hydroxy-uridine, and its close analogue 5-methoxy-uridine, an influence of the resonance between the valence π electrons of O(5) and the pyrimidine ring (Fig. 2) is reflected by a considerably shorter C(5)–O(5) bond (1.359 and 1.358 Å, respectively) than C(7)–O(7) bond in the –CH_2OH group of 5-hydroxymethyl-2′-deoxyuridine (for two sites of O(7): 1.407 and 1.395 Å), as well as C(7)–O(7) in the –CH_2OCH_3 group of 5-methoxymethyl-2′-deoxyuridine (1.438 Å). The last two

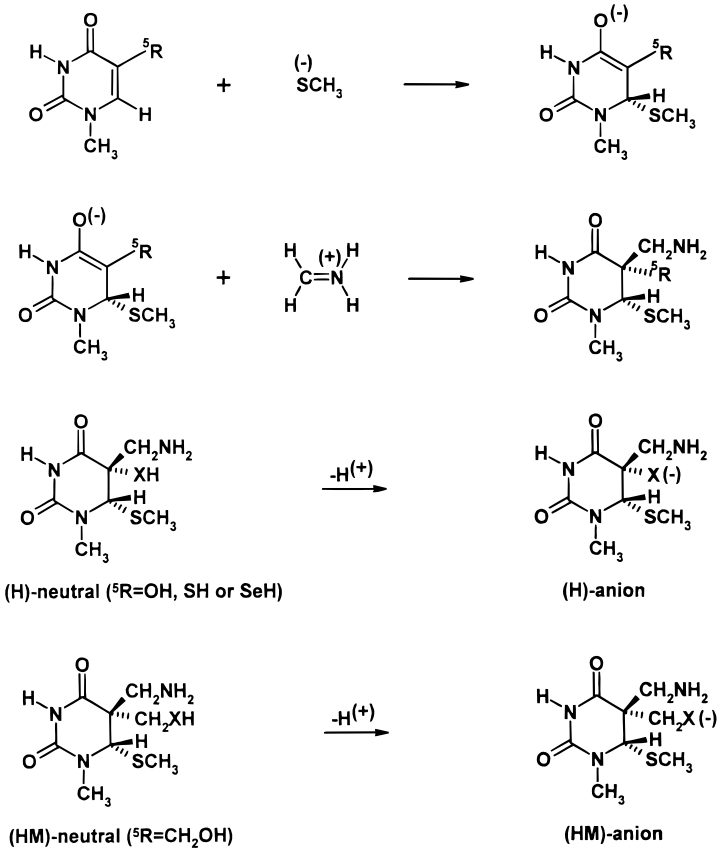

FIG. 1. The top row shows modeling of nucleophilic attack ($SCH_3^{(-)}$ simulating cysteine residue in thymidylate synthase active center) on the substrate analogue (^5R-1-methyluracil, ^5R = OH, CH_2OH, SH, or SeH, simulating oh^{5}dUMP, hm^{5}dUMP, sh^{5}dUMP, or seh^{5}dUMP, respectively). The second row corresponds to cofactor attachment at C(5), modeled by the methyleneimine cation reaction with the incipient enolate of the former reaction. The bottom two rows represent the proton release from model ternary complexes with ^5R = OH, CH_2OH, SH, and SeH.

substituents cannot induce the resonance because of the screening by the $-CH_2-$ group; therefore both C(7)–O(7) bonds may be considered strictly single. The C(5)–O(5) bond in each of the former two structures is by confrontation partly double. Figure 3 shows 12 possible resonance structures of the uracil ring in 5-hydroxyuridine (oh^{5}Urd), between them those (printed bold) with the C(5) and O(5) connected by double bonds and with a single positive charge on the hydroxylic O(5). The structures in the latter group tend obviously to promote dissociation of the proton from the hydroxylic group. Their total contribution to the hybrid of all possible resonance structures amounts to 27.7%, as estimated by the HOSE (harmonic oscillator stabilization energy) method (*11*).