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Relationship between structure and antiviral activity of 5-methoxymethyl-2′-deoxyuridine and 5-methoxymethyl-1-(2′-deoxy-β-D-*lyxo*furanosyl)uracil

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Summary

5-Methoxymethyl-1-(2′-deoxy-β-D-*lyxo*furanosyl)uracil (MMdLU) was not active against the herpes simplex viruses. The relationship between molecular conformation and antiviral activity for the two epimers, 5-methoxymethyl-2′-deoxyuridine (MMdUrd) and MMdLU, is discussed. MMdUrd was phosphorylated by the virus-induced deoxythymidine kinase. In contrast, MMdLU did not serve as a substrate for the kinase. The geometry and distance between the 5′-CH_2OH and 3′-OH groups of the furanose ring appear to be key factors in determining the efficiency of phosphorylation by the virus-induced deoxythymidine kinase, and hence antiviral activity.

5-Methoxymethyl-2′-deoxyuridine; Deoxythymidine kinase; 3′-Epimer; Molecular conformation

Introduction

During the last few years several selective antiherpes agents have been developed [6,7,11,15,19]. In order to understand the molecular basis for selective action

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of antiherpes drugs several approaches can be used. (i) Synthesize analogues and relate the structural modifications to antiviral activity [4,8,14,16–18,22,23]. The major limitation of this approach is that it does not provide information regarding the stereochemical requirements for biological activity. (ii) Determine the three dimensional structure of the enzyme substrate complex of viral deoxythymidine kinase, which is responsible for 'activation' of these drugs [7,25]. However, further progress using this approach must await elucidation of the structure of the enzyme–substrate complex in the crystalline state. (iii) Undertake three dimensional structural studies on nucleoside analogues. These latter studies are feasible because nucleoside analogues can be obtained in the crystalline state for x-ray crystallography and their molecular conformation in aqueous solution can be determined by NMR spectroscopy. The correlation of their molecular conformation with biological activity should provide information concerning the conformation required for binding at the active centre of the enzyme. In this communication, the relationship between molecular conformation, antiviral activity and interaction with virus-induced deoxythymidine kinase for 5-methoxymethyl-2′-deoxyuridine (MMdUrd) and its 3′-epimer, 5-methoxymethyl-1-(2′-deoxy-β-D-*lyxo*furanosyl)uracil (MMdLU) will be discussed.

Experimental

(i) Viruses

Herpes simplex type 1 (HSV-1) strains F (ATCC VR-733) and McIntyre (ATCC VR-359) were obtained from the American Type Culture Collection (Rockville, MD). The strain (KOS) was obtained from Dr. Rawls, Baylor University, College of Medicine (Houston, TX). The thymidine kinase-deficient (TK^-) of HSV-1 strains B2006 [5] and Cl (101) [13] were provided by Dr. Y.C. Cheng (University of North Carolina, Chapel Hill, NC, U.S.A.) and Dr. H. Field (Department of Pathology, University of Cambridge, Cambridge, England), respectively. The conditions for the preparation of virus stocks have been described [2,3,9].

(ii) Drug inhibition assays

For antiviral assays, confluent monolyers of primary rabbit kidney (PRK) cell cultures were infected with 100 plaque forming units per well in a microtitre tray [2,9]. The virus was allowed to adsorb for 1 h at 37°C, and immediately thereafter, exposed to varying concentrations of the test compounds in Eagle's minimal essential medium (EMEM) containing 3% fetal calf serum (FCS). Plaques were allowed to develop for 72 h before fixation, staining and enumeration. From dose-response curves, the concentration required to reduce the number of plaques by 50% (ID_{50}) was determined.

(iii) HSV-1 deoxythymidine kinase (TK) activity measurements

Deoxythymidine kinase-deficient (TK^-) HeLa cells were originally developed by S. Kit and obtained from Dr. Y.-C. Cheng. The TK^- HeLa cells were grown in

TABLE 1

Antiviral activity of BVdUrd, MMdUrd and MMdLU against strains of herpes simplex virus type 1 (HSV-1) in primary rabbit kidney cell cultures

Virus strain	Minimum inhibitory concentration (μg/ml)[a]		
	BVdUrd	MMdUrd	MMdLU
KOS	0.02	10	>400[b]
F	0.02	20	>400
McIntyre	0.02	40	>400
B2006 (TK^-)[c]	300	>400	>400
Cl (101) (TK^-)[c]	150	>400	>400

[a] Concentration required to cause 50% reduction in viral cytopathogenicity.
[b] No activity up to 400 μg/ml (highest concentration tested).
[c] Deficient in deoxythymidine kinase activity.

EMEM supplemented with 10% FCS. For induction of virus-specified TK, confluent TK^- HeLa cells were infected with HSV-1 (strain KOS) at a multiplicity of $10^{6.2}$ $CCID_{50}$ (cell cultures 50% infective dose) per ml (5ml per 225 cm^2 Roux bottle). After 1 h virus adsorption, 95 ml EMEM + 1% FCS were added, and the cells were further incubated for 2 days at 37°C. At that time viral cytopathogenicity had reached about 50%. The cell cultures were washed 3 times with 50 mM Tris-HCl, pH 8.0, in 0.9% NaCl, and frozen at −20°C. After thawing, the cells were suspended in 0.1 M Tris-HCl, pH 8.0, containing 20 mM β-mercaptoethanol. Following sonication (2 times, 10 sec), the cell homogenate was cleared by centrifugation for 30 min at 70000 × *g* and the supernatant was divided in aliquots and stored at −70°C until used.

The standard HSV-1 TK assay mixture contained 2 mM ATP, 2 mM $MgCl_2$, 7.5 mM NaF, 7.25 mM phosphoenolpyruvate, 78 μg/ml pyruvate kinase, 1.6 mM dithiothreitol, 0.121 μCi/25 μM 2′-deoxy-[2-^{14}C]-thymidine, varying concentrations (5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 500 or 1000 μM) of the test compounds (MMdUrd, MMdLU or BVdUrd), 0.78 mg/ml albumin, and 15 μl enzyme extract in a total volume of 90 μl 135 mM Tris-HCl, pH 7.5. The assay mixtures were incubated at 37°C for 60 min, and the reaction was terminated by chilling the samples to 0°C in an ice bath. Of the samples 50-μl volumes were applied onto Whatman DE81 discs and washed 3 times with ethanol. The discs were then dried and evaluated for radioactivity in a xylene-based scintillant (mixture of 0.2 ml water, 1 ml Soluene and 10 ml Lipoluma).

Results and Discussion

The antiviral activity of MMdUrd and MMdLU was determined against HSV-1. (*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (BVdUrd) and MMdUrd were included as positive controls. MMdLU was not active against HSV-1 (Table 1). The loss of activity against HSV-1 following inversion of the 3′-position has also been re-

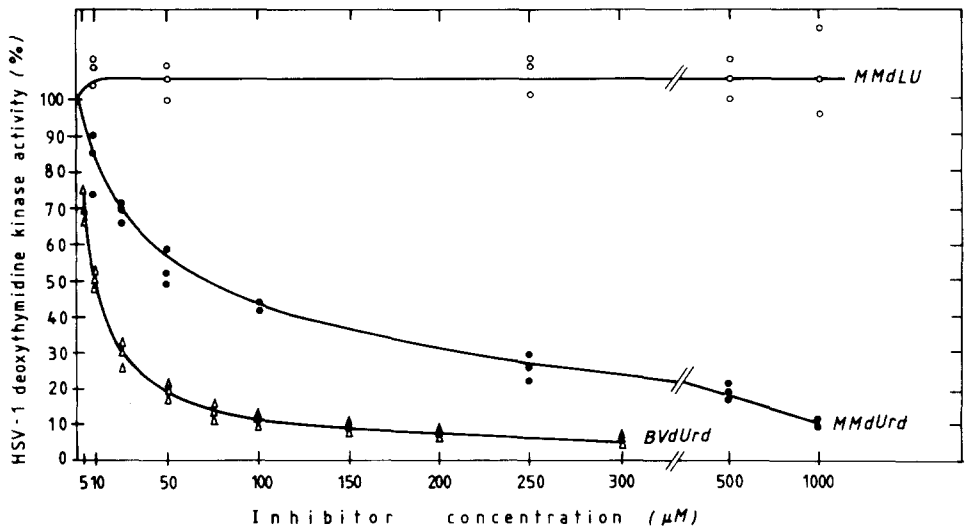

Fig. 1. Interaction of MMdLU (○), MMdUrd (●) and BVdUrd (△) with the phosphorylation of [2-^{14}C]dThd by HSV-1-specified dThd kinase.

ported for the epimers, BVdUrd and (*E*)-5-(2-bromovinyl)-1-(2′deoxy-β-D-*lyxo*-furanosyl)uracil (BVdLU) [4,25].

BVdUrd showed high affinity for the HSV-1-specified dThd kinase: its IC_{50} for the enzyme was about 10 μM. When assayed under the same conditions, MMdUrd showed an IC_{50} of about 70 μM (Fig. 1). In marked contrast with MMdUrd, MMdLU did not interact with the HSV-1 dThd kinase even at 1000 μM (the highest concentration tested), there was not the slightest competition with the phosphorylation of 2′-deoxy-thymidine (IC_{50} >> 1000 μM). The lack of phosphorylation may account for the loss of antiviral activity when the configuration of the 3′-position is inverted.

The structural formulae and the three dimensional conformations in the crystal state for MMdUrd and MMdLU are shown in Fig. 2. In order to get a better understanding of the relationship between antiviral activity and molecular structure, the two molecules were compared using the computer program PROFIT [21]. The planar pyrimidine ring of MMdUrd was superimposed on that of MMdLU and the distances between equivalent atoms in each molecule were calculated (Table 2). The stereoscopic representation of the superimposed molecules is shown in Fig. 3. Both compounds have similar *anti*-glycosidic linkages [12,20]. Inversion of the 3′-OH group from the *exo* to the *endo* position changes the puckering of the furanose ring, alters the conformation of the side chains at C(5′) of the sugar ring and at C(5) of the pyrimidine ring. MMdUrd has C(2′)-*endo* puckering of the furanose ring. In contrast, MMdLU has C(4′)-*exo* puckering. The exocyclic C(5′) side chain exists predominantly in the g^+ conformation in MMdUrd and primarily in the t conformation in MMdLU (Fig. 4). The torsion angles C(3′)-C(4′)-C(5′)-O(5′) and O(4′)-C(4′)-C(5′)-O(5′) are +55.9° and −63.1° for MMdUrd and the correspond-

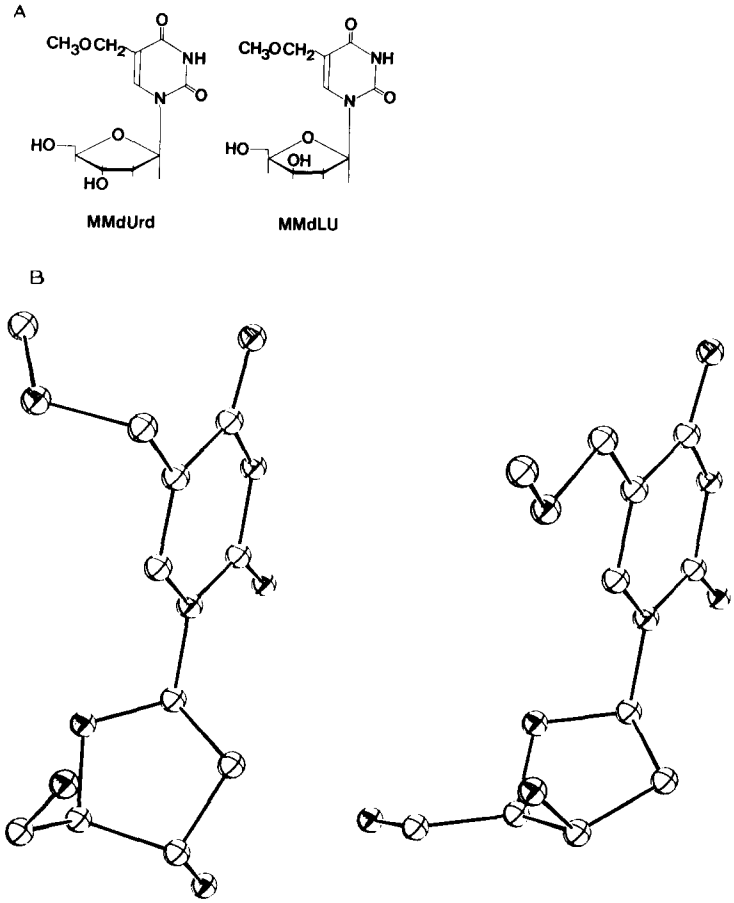

Fig. 2. (A) Structure of 5-methoxymethyl-2′-deoxyuridine (MMdUrd) and 5-methoxymethyl-1-(2′-deoxy-β-D-lyxofuranosyl)uracil (MMdLU). (B) Three dimensional view of MMdUrd and MMdLU. Hydrogen atoms are not shown for the sake of clarity.

ing angles in MMdLU are −173.4° and +70.1°. The orientation of the side chain at the 5-position of the pyrimidine ring is also different in these molecules. This side chain is almost perpendicular to the plane of the pyrimidine ring in MMdUrd and is essentially in the same plane as the pyrimidine ring in MMdLU (Fig. 2B).

The structure in the crystalline state represents a thermodynamically stable conformation. However, in aqueous media, the molecular conformation is less rigid and the conformational flexibility of the molecule is determined by NMR spectroscopy. The conformation of the furanose ring of nucleosides has been investigated within the pseudorotational concept [1]. Each puckered conformation is unequivocally described by two pseudorotational parameters, the phase angle (P) and the degree of pucker (τ_m). A potential energy barrier to free rotation due to

TABLE 2

Comparison of MMdUrd and MMdLU distances between equivalent atoms when the pyrimidine ring of MMdUrd is superimposed on the pyrimidine ring of MMdLU

MMdUrd – MMdLU	Distance (nm)
N(1) . . .N(1)	0.35
C(2) . . .C(2)	0.21
0(2). . . .O(2)	0.28
N(3) . . .N(3)	0.10
C(4) . . .C(4)	0.18
O(4) . . .O(4)	0.36
C(5) . . .C(5)	0.19
C(5,1). .C(5,1)	0.53
O(5,2) .O(5,2)	16.8
C(5,3). .C(5,3)	36.4
C(6) . . .C(6)	0.29
C(1'). . .C(1')	0.37
C(2'). . .C(2')	3.9
C(3'). . .C(3')	12.5
O(3') . .O(3')	40.6
C(4'). . .C(4')	4.8
O(4') . .O(4')	3.7
C(5'). . .C(5')	16.4
O(5') . .O(5')	43.0

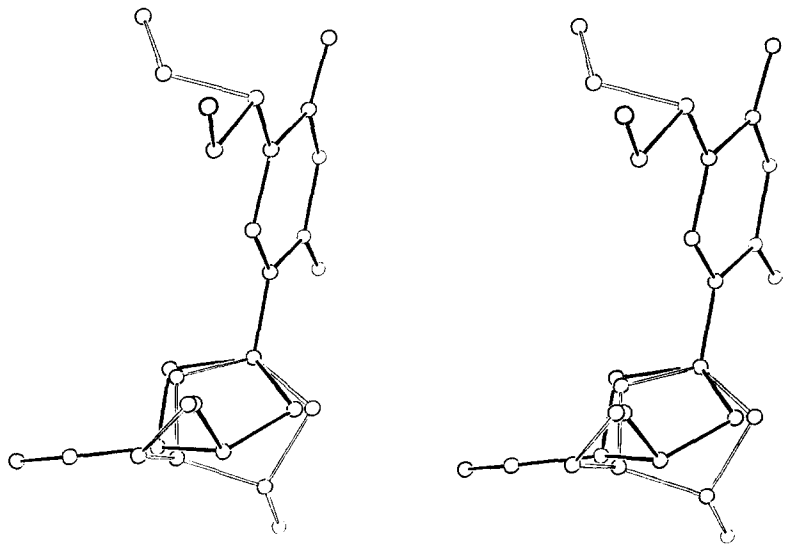

Fig. 3. Stereoscopic view of the superimposed molecules MMdUrd (open bonds, o=o) and MMdLU (solid bonds, o—o). Hydrogen atoms are not shown for the sake of clarity.

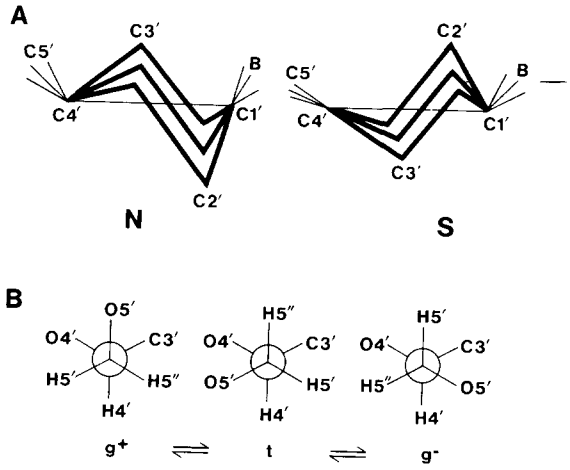

Fig. 4. (A) Schematic drawing showing fluctuations in the two predominant pseudorotational modes of the furanose ring of nucleosides in solution. (B) Neuman projections of the staggered conformations about the C(5′)-C(4′) bond of the furanose ring.

substituents on the furanose ring causes most nucleosides to fall within two relatively narrow ranges of the phase angle: N-conformers where P ranges from 3° to 23° and S-conformers where P ranges from 139° to 175°. The fluctuations of these two conformational states are shown in Fig. 4A. MMdUrd exists primarily in the S-state (60%) while the 3′-epimer, MMdLU, exists almost entirely in the N-state (97%). The exocyclic C(5′) side chain is free to rotate from one staggered conformation to another as shown in Fig. 4B. The preferred conformation for MMdUrd is the g^+ state (61%), whereas MMdLU has a 57% population in the t state. The strong destabilization of the g^+ mode in MMdLU is most likely due to the steric effects of the cisodial configuration. In MMdUrd, the methoxymethyl group at C(5) does not seem to have any preference for a particular orientation relative to the pyrimidine ring. However, since anisotropic effects are of greater magnitude in MMdLU, the preferred conformation is such that the methoxy group is closer to the sugar moiety. This is due to increased interaction of the side chain with the *endo* 3′-OH group. The preferred molecular conformations for MMdUrd and MMdLU determined in solution are similar to the molecular conformations found in the crystal state.

Phosphorylation of MMdUrd to the 5′-monophosphate by the viral kinase is an essential step for antiviral activity [24]. MMdLU failed to show affinity for the viral enzyme (Fig. 1) and this probably accounts for the lack of antiviral activity. In the crystal state, the position of the *endo* 3′-OH group of MMdLU is very close (3,3 nm) to the corresponding position of the 5′-OH group of MMdUrd as shown in the stereoscopic view of the superimposed molecules (Fig. 2). In aqueous solution, even though the molecule has conformational flexibility, the cisoidal relationship between the 3′-OH and 5′-CH_2OH groups in MMdLU hinders the 5′-CH_2OH group from acquiring the orientation necessary for phosphorylation at the active

centre of the enzyme. Thus geometry and distance between the 5′-CH_2OH group and the 3′-OH group of the furanose ring appear to be key factors in determining the efficiency of phosphorylation by the kinase. Further studies to correlate the conformation of other nucleoside analogues with antiherpes activity are in progress to test this hypothesis.

Acknowledgements

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