Structural Requirements for the Inhibition of Uridine-Deoxyuridine Phosphorylase by Thymine Nucleosides Containing an Unnatural Carbohydrate Moiety

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SUMMARY

1-(2′-Deoxy-β-D-xylopyranosyl)thymine (β-II) produces as great an inhibitory effect on uridine-deoxyuridine phosphorylase as 1-(2′-deoxy-β-D-glucopyranosyl)thymine (β-I), whereas the inhibitory action of 1-(2′-deoxy-β-D-xylofuranosyl)thymine (V) is only 1/25 that of the above-mentioned β-nucleosides. The inhibitory activity of 1-(2′-deoxy-α-D-xylopyranosyl)thymine (α-II) is equal to that of 1-(2′-deoxy-α-D-glucopyranosyl)thymine (α-I), both being less effective than the corresponding β-anomers and more active than V. α- and β-1-(2′-Deoxy-D-ribopyranosyl)thymine (α-III and β-III) are noninhibitory under the same conditions. Hence, both the presence of a pyranose lactol ring and the *xylo* configuration of the hydroxyl group C-3′ are necessary for the inhibitory action of these compounds. 1-(Tetrahydro-2′-pyranyl)thymine (VI) is not active.

INTRODUCTION

The synthetic pyrimidine nucleoside, 5-fluoro-2′-deoxyuridine (FUDR), causes tumor regression in advanced human cancer patients (1), and 5-iodo-2′-deoxyuridine (IUDR) has a radiosensitizing effect *in vitro*. The therapeutic usefulness of both these compounds is diminished by their rapid cleavage to the respective free bases in human and animal tissues (1–4). Degradation of these compounds is effected by two different enzymes, thymidine phosphorylase (EC 2.4.2.4) (5) and uridine-deoxyuridine phosphorylase (EC 2.4.2.3) (6, 7).

Previously, we reported that the unnatural nucleoside 1-(2′-deoxy-β-D-glucopyranosyl)thymine (β-I, Fig. 1) (8, 9) is a powerful competitive inhibitor of uridine-deoxyuridine phosphorylase *in vitro* (10) and is also effective *in vivo*. This compound, given to cats together with 5-iodo-2′-deoxyuridine, causes a considerable increase in the incorporation of IUDR into the DNA of intestine, spleen, and bone marrow (11, 12). The α-anomer (α-I) is less active in this respect.

The high affinity of β-I toward this enzyme is surprising, as its carbohydrate moiety differs markedly from that of the natural nucleosides such as thymidine (IV) in a variety of ways: the sugar contains one more carbon atom, the hydroxyl group at C-3′ has the opposite configuration, and the ring has a pyranose, rather than a furanose, structure. With the exception of some antibiotics such as amicetin (13), blasticidin (14, 15), and gougerotin (16), simple pyrimidine nucleosides with a pyranose carbohydrate moiety have hitherto been regarded as biologically inactive.

In order to investigate whether the inhibitory action of β-I is dependent on the presence of one of these three different structural characteristics, or on a combination of them, several thymine 2′-deoxynucleosides, different from thymidine (IV) in one or two of the structural features

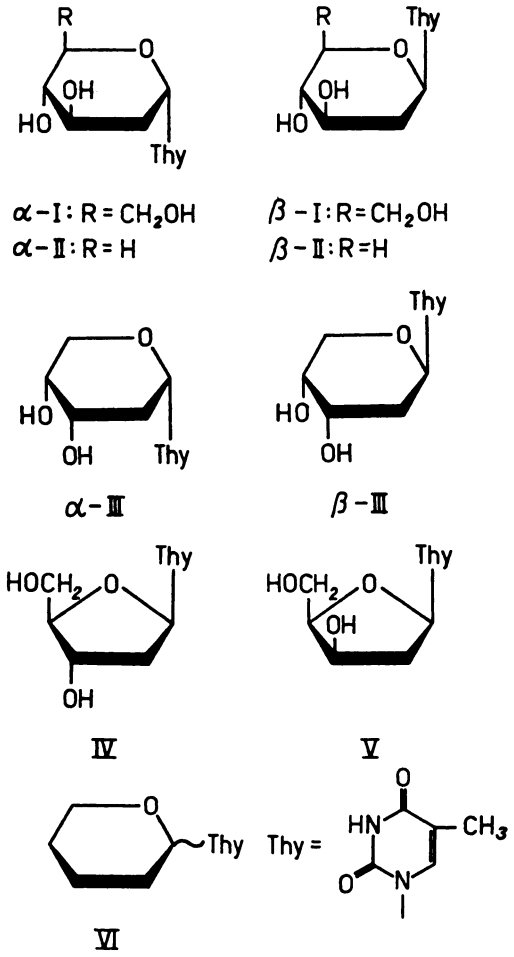

FIG. 1. *Thymine nucleosides of 2-deoxy-D-glucopyranose (α-I, β-I); 2-deoxy-D-xylopyranose (α-II, β-II); 2-deoxy-D-ribopyranose (α-III, β-III); 2-deoxy-β-D-ribofuranose (IV), 2-deoxy-β-D-xylofuranose (V), and tetrahydropyran (VI)*

mentioned, were synthesized and tested for enzyme inhibitory activity.

MATERIALS AND METHODS

The anomers of 1-(2′-deoxy-D-glucopyranosyl)thymine (α-I, β-I) (9) and 1-(2′-deoxy-D-ribopyranosyl)thymine (α-III, β-III) (17)[1] were prepared as described previously.

α- and β-1-(2′-deoxy-D-xylopyranosyl)-thymine (α-II, β-II), two hitherto unknown compounds, were obtained by condensation of monothyminylmercury with 2-deoxy-3,4-di-*O*-acetyl-D-xylopyranosyl chloride and separated by cellulose column chromatography (18). The pyranose structure of α-II and β-II was established by their consumption of one mole of sodium periodate. The configuration at C-1′ was determined by comparison of the optical rotations of the enantiomorphic nucleoside dialdehydes of α-II ($[\alpha]_D^{20} - 68°$) and β-II ($[\alpha]_D^{20} + 71°$) with those of α-III ($[\alpha]_D^{20} - 64°$) and β-III ($[\alpha]_D^{20} + 69°$), which were obtained after periodate oxidation of the anomeric nucleosides. The configurations of α-III and β-III, furthermore, have been deduced from their infrared spectra (17) and have more recently been proved by the stereospecific formation of an 2,3′-anhydronucleoside from 3′,4′-dimesyl- and 3′,4′-carbonato-β-III (19). In agreement with conclusions drawn from inspection of the molecular models, attempts to cyclize the corresponding derivatives of α-III failed. The alkaline cleavage of the anhydronucleoside gives β-II.

1-(2′-Deoxy-β-D-xylofuranosyl)thymine (V) was obtained by alkaline cleavage of 2,3′-anhydrothymidine (20, 21). 1-(Tetrahydro-2′-pyranyl)thymine (VI) (22) was kindly supplied by Professor C. C. Cheng, Midwest Research Institute, Kansas City. The anomeric configuration of this compound is unknown.

The purity of all compounds was checked by thin-layer chromatography on silica gel HF_{254} (Merck) with several solvent systems using ultraviolet light and conc. H_2SO_4 for localization of the spots.

A uridine-deoxyuridine phosphorylase preparation was obtained by the treatment of an acetone powder from Ehrlich ascites carcinoma cells with 15 volumes of 0.05 M Tris buffer pH 7.4, for 1 hr at 0° and subsequent centrifugation at 100,000 *g* for 1 hr. The effects of the various thymine nucleosides on the enzymatic arsenolysis of 1 mM deoxyuridine was investigated by a previously described technique (10). The extract from the ascites tumor cells was used without further purification, since under the conditions of the assay deoxyuridine can only undergo degradation.

[1] Also G. Etzold, R. Hintsche and P. Langen, *Chem. Ber.* in press.

Phosphorylation to the nucleotide, the other biochemical reaction open to deoxyuridine, requires large amounts of ATP or even an ATP regenerating system, and probably could not proceed in arsenate buffer. The extent of deoxyuridine cleavage was followed by the diphenylamine reaction (23). Under the conditions of the assay, liberation of 1 μmole of deoxyribose corresponded to an extinction of 0.835 at 601 mμ.

RESULTS AND DISCUSSION

To determine the significance of the number of carbons in the carbohydrate moiety, the inhibitory effect of the anomers of 1-(2′-deoxy-D-xylopyranosyl)thymine (α-II and β-II) on uridine-deoxyuridine phosphorylase was compared with that of the 1-(2′-deoxy-D-glucopyranosyl)thymine anomers (α-I and β-I). α-II and β-II differ from α-I and β-I only in having no terminal CH_2OH group (Fig. 1). It is apparent from Fig. 2 that the pentapyranosides, α-II and β-II, possess an activity similar (α-anomer) or equal (β-anomer) to that of the corresponding hexosides α-I and β-I. Therefore, the affinity toward the enzyme is not affected by changes in the number of carbons in the sugar residue.

To study the effect of ring contraction from pyranose to furanose on the inhibition of the enzyme, β-II and 1-(2′-deoxy-β-D-xylofuranosyl)-thymine (V) were compared. Both have the same configuration at the asymmetric C-atoms and differ only in the lactol ring size (Fig. 1). As can be seen from Fig. 2, conversion of the pyranose to the furanose structure leads to a marked loss of inhibitory activity. The concentration required for 50% inhibition of deoxyuridine cleavage is 25 times greater with V than with β-II.

Finally, in order to elucidate the importance of the configuration of the hydroxyl group at C-3′ of the sugar component, we investigated the inhibitory action of the α- and β-anomers of 1-(2′-deoxy-D-ribopyranosyl)thymine (α-III and β-III) that differ from the active isomers α-II and β-II only in having the opposite configuration of the OH group attached to the C-3′ (Fig. 1). As already

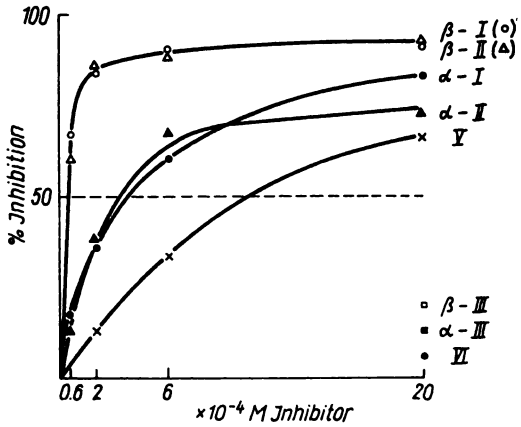

FIG. 2. *Influence of various unnatural thymine nucleosides on the enzymic arsenolysis of deoxyuridine at various inhibitor concentrations*

Reaction mixture: 120 mM arsenate; 200 mM Tris-HCl pH 7.4; 1 mM deoxyuridine; 0.1 ml enzyme solution. Final volume 0.5 ml. The reaction was started by the addition of the enzyme. Incubation: 1 hr at 37°. The deoxyuridine cleavage in the uninhibited sample was 0.245 μmole.

mentioned (17), it is surprising that both anomers, α-III and β-III, were found to possess only a very weak inhibitory activity. (Fig. 2). The concentration required for 20% inhibition is 100-fold higher with these anomers than with the anomers of I or II.

As found by Zimmerman (24), the pyranose thymine nucleosides of 2-deoxy-D-allose (25) and digitoxose (8), which have a structure similar to III, do not inhibit the phosphorylase. However, their anomeric configuration was not determined. The sugar of these nucleosides contains one more carbon atom, whereas the configuration of the hydroxyl groups attached to the ring is the same as in III. In that case also, no significant difference in the inhibitory action between the pentose and hexose homologs is found.

None of the unnatural thymine nucleosides investigated here serve as a substrate for uridine-deoxyuridine phosphorylase, although, for example, β-III is distinguished from the natural substrate thymidine (IV) only by a lactol ring of different size, and V differs from IV only by having an opposite configuration of the OH group at C-3′.

It is obvious from the structure-activity correlations described above that thymine-2′-deoxynucleosides with the D-configuration of the OH group at C-4′ develop a maximum inhibitory action only if the OH group at C-3′ possesses the *xylo*-configuration, and the structure of the lactol ring is pyranoid. It is perhaps surprising that the conversion of the D-xylose configuration to the D-ribose configuration, which makes the inhibitor structurally more similar to the natural substrate, does not result in an increase, but rather in a nearly complete loss of inhibitory activity. One possible interpretation of our results could be that the pyranose ring is a basic requirement for an inhibitory effect on this enzyme. In III, however, due to the *ribo*-configuration of the 3′-OH group, the pyranose ring might be sterically hindered from exerting its activity. Another explanation could be that the *xylo*-configuration of the 3′-OH group is decisive for the blockade of the enzyme, possibly due to the formation of a hydrogen bond to the enzyme. The approach to the enzymic binding site, however, might be for stereochemical reasons only possible with a pyranose structure, which is more readily deformable than the furanose structure. In order to distinguish between these two possibilities we plan to investigate the corresponding pyranose thymine deoxynucleosides in which the 3′-OH group has been eliminated.

1-(Tetrahydro-2′-pyranyl)thymine (VI), which may be regarded as a pyranoid thymine nucleoside of a 2,3,4-trideoxypentose, is practically without inhibitory activity (Fig. 2). This suggests that the inhibitory effect is due to the presence of the OH group attached to the C-4′ in a suitable configuration. We intend to determine the influence of this hydroxyl group on the inhibitory activity of these compounds by a systematic study of the structure-activity correlations with suitable compounds.

All the unnatural nucleosides so far investigated inhibit only the uridine-deoxyuridine phosphorylase and do not influence the thymidine phosphorylase. Since it is this latter enzyme that splits FUDR and IUDR in human tissues, its inhibition would be of particular practical importance. Further studies on the interrelationships between the carbohydrate structure and inhibitory activity may be helpful in finding unnatural thymine nucleosides that would also inhibit thymidine phosphorylase.

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