X-Ray crystal structure of sangivamycin, a potent inhibitor of protein kinases

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The X-ray crystal structure of sangivamycin, a potent nucleoside inhibitor of protein kinases, has been determined. Sangivamycin crystallizes from water with its purine ring in a conformation anti to its ribose sugar. Such an anti conformation has been detected in solution for sangivamycin and other potent protein kinase inhibitors and appears to correlate with inhibitor potency [(1990) Biochemistry (in press)]. An intramolecular hydrogen bond between purine ring substituents is detected in the X-ray structure and may be an important structural feature of sangivamycin related to its degree of inhibition of rhodopsin kinase and of protein kinases C and A.

Inhibitor; Protein kinase; Nucleoside

1. INTRODUCTION

Inhibitors are very useful tools for studying the role and function of enzymes. In order to design the most potent and specific inhibitors, information about inhibitor structure is necessary. Such structural information should serve as a guide for development of even more potent and selective inhibitors.

Protein kinases catalyze phosphotransfer reactions from an ATP/GTP donor to a protein substrate. These enzymes are involved in most cellular, metabolic, transduction and proliferation processes.

Inhibitors of protein kinases can be divided into two classes: those that compete with the protein substrate (either as an integral part of the enzyme, or independently, (e.g. see [2–4]); and those that compete at the ATP/GTP binding site [5–10].

Sangivamycin inhibits protein kinases, including nuclear protein kinases [11], protein kinases A and C [12], and rhodopsin kinase [1]. This naturally occurring antibiotic (synthesized by Streptomyces rimosus) also has significant antitumor activity against L 1210 leukemia in mice [13].

Sangivamycin assumes predominantly the anti conformation (sugar anti to the base) in solution as determined by Nuclear Overhauser Effect measurements. This anti conformation appears to be required for efficient nucleoside binding to rhodopsin kinase [1] and presumably to other protein kinases. In this report we present the solid state structure of sangivamycin.

2. MATERIALS AND METHODS

Sangivamycin was obtained from the National Institutes of Health, National Cancer Institute (Natural Products Branch) Division of Cancer Treatment.

Crystals of sangivamycin monohydrate were grown from aqueous solution by slow evaporation in the open atmosphere. Crystal data: 

C12H18N4O5 · H2O, M_r = 328.1, orthorhombic, a = 11.694 (3) Å, b = 17.519 (4) Å, V = 1388 Å³, Z = 4, D_{calc} = 1.571 g·cm⁻³, μ (Mo Kα radiation) = 1.19 cm⁻¹. Space group P2₁2₁2₁ from systematic absences. Crystal dimensions 0.2 x 0.1 x 0.1 mm.

Intensity data to θ = 23° were collected with an Enraf-Nonius CAD-4 diffractometer using ω/2θ scans. A total of 1164 reflections were recorded and the usual Lorentz and polarization corrections were applied [14]. The unit cell parameters were calculated from the diffractometer setting angles for 25 general reflections.

The crystal structure was solved by direct methods using the SHELXS-86 program [14]. Approximate coordinates for the non-hydrogen atoms of the aromatic rings were obtained from an E-map. The rest of the molecule was found in subsequent difference Fourier maps and their positions were refined with the oxygen-hydrogen distance restrained to 1.08 Å. The refinement converged at R = 0.045 and R_w = 0.042 where R = ∑ |F_o| - |F_c|)/∑ |F_o| and R_w = ∑w(|F_o| - |F_c|)²/∑w|F_c|² while w = 1/σ²(F)+0.0004 F². The refinement was carried out using the SHELX-76 program. The values of interatomic distances, bond angles, and torsion angles are available from the authors.

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Fig. 1. Solid-state conformation of sangivamycin in crystals of monohydrate. C, O and N are labeled, H atoms are represented by small circles. A hydrogen bond is marked with a broken line.

3. RESULTS

The molecular structure of sangivamycin is shown in Fig. 1. The most significant finding is that the furanoside ring assumes an anti conformation with respect

to the base, a conformation which has been detected in solution [1]. The fractional coordinates and estimated standard deviations are presented in Table I.

The furanoside ring has an envelope conformation with C(3') displaced by 0.48 Å from the least squares plane through the other four ring atoms. The plane is almost perpendicular (85°) to the best plane through the aromatic rings. Steric hindrance between the amido group (N10) on C6 and the amino group (C11, O12, N13) on C7 is alleviated by a 30° rotation of the amide group from the purine plane. One of the hydrogen atoms of the amino group forms an intramolecular H-bond with the oxygen of the amide moiety.

As a stereo view of the packing arrangement of crystalized sangivamycin is presented in Fig. 2. The condensed rings are stacked along the c-axis (Fig. 2) with the 5-membered ring sandwiched between the 6-membered ring and vice versa. The shortest interplanar interatomic distance at 3.30 Å occurs between C(4) and C(6).

Sangivamycin and water molecules are associated by an extensive network of hydrogen bonds involving the two amino and 3 hydroxy groups and both water molecules (Table II).

4. DISCUSSION

Sangivamycin is the most potent inhibitor of rhodopsin kinase and one of the most effective blockers of protein kinase C [1,12]. In solution, sangivamycin assumes predominantly the anti conformation, as determined by Nuclear Overhauser measurements. This conformational property is shared by other kinase nucleoside inhibitors and is lacking in compounds that are poor inhibitors, and therefore may be related to its inhibitory properties [1].

In the solid state, sangivamycin also exists in the anti conformation. Moreover, our studies reveal that the N(10) amine is involved in an intramolecular hydrogen bond with the carbonyl residue of the amide group at C(7), a bond planar with the base. This feature of the intermolecular hydrogen bond may help explain why the C10 amide yields a more potent inhibitor for rhodopsin kinase than the C10 nitrile, amidine or amidoxime [1] and suggests a structure to mimic in the search for even more potent inhibitors. The amide group at C(7) also may interact via hydrogen bond with an enzyme. In addition, the conformation of the ribofuranoside can play an important role in protein-nucleoside recognition. Newman projection reveals that sangivamycin assumes trans-gauche conformation about the backbone C(5')-O(5') bond and gauche-gauche staggered conformation about the exocyclic C(4')-C(5') bond, as suggested for nucleosides with anti sugar-base torsion preferred in the anti position [15].
Fig. 2. Stereoview of the packing of sangivamycin molecules in the crystals seen approximately along the c-axis.

Table II

Hydrogen-bonded distances for sangivamycin monohydrate

<table>
<thead>
<tr>
<th>D-H...A</th>
<th>D-A (Å)</th>
<th>H...A (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(10)-H(4)....O(12)</td>
<td>2.88(1)</td>
<td>1.90</td>
</tr>
<tr>
<td>N(13)-H(5)....O(8')^II</td>
<td>3.00(1)</td>
<td>2.12</td>
</tr>
<tr>
<td>N(13)-H(6)....O(W)^IV</td>
<td>3.20(1)</td>
<td>2.12</td>
</tr>
<tr>
<td>O(7')-H(7')....O(12)^III</td>
<td>2.68(1)</td>
<td>1.74</td>
</tr>
<tr>
<td>O(8')-H(8')....N(3)^IV</td>
<td>2.95(1)</td>
<td>1.91</td>
</tr>
<tr>
<td>O(9')-H(9')....O(W)^V</td>
<td>2.68(1)</td>
<td>1.62</td>
</tr>
<tr>
<td>O(W)-H(IW)....O(9')^v</td>
<td>2.71(1)</td>
<td>1.72</td>
</tr>
<tr>
<td>O(W)-H(2W)....N(1)^v</td>
<td>2.80(1)</td>
<td>1.80</td>
</tr>
</tbody>
</table>

Roman numeral superscripts refer to atoms in the following equivalent positions:

^I 0.5-x, -y, -0.5+z

^II 0.5-x, -y, 0.5+z

^III 1-x, -0.5+y, -0.5-z

^IV 0.5 +x, 0.5-y, -z

v x, y, -1+z

REFERENCES


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