

A Quantum-Chemical Model of the Inhibition HIV-1 Integrase Action by Iodine Complex Compounds and Lithium Halogenides

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Abstract

The water-nucleotide AGA triplet - LiCl(I)-I₂-α-dextrin-peptide system was investigated using UV spectroscopy. The good agreement between the experimental UV-frequencies of the system with the theoretical frequencies for LiCl(I)-I₂-α-dextrin-nucleotide complexes indicates that when interacting with LiCl(I)-I₂-α-dextrin complexes AGA triplet nucleotides displace peptide and form a new complex with iodine and lithium halogenides.

Using the quantum-chemical DFT it was shown that the LiCl(I)-I₂-α-dextrin-nucleotide complexes inhibit the activity of the catalytic site of integrase inside the PIC. They disrupt the conditions ensuring the occurrence of catalytical cleavage of the phosphoester bond in the phosphate group of the viral DNA by the active catalytic site of integrase as they form a nucleoprotein complex consisting of the nucleotides of the viral DNA, LiCl(I)-I₂-α-dextrin complexes and active components of the catalytic site of integrase. In these complexes magnesium ions are coordinated by the carbonyl oxygen atom of thymine rather than by the oxygen atoms of the phosphate group.

The effect of antiviral action of LiCl(I)-I₂-α-dextrin-peptide complexes against HIV-1 has been established on a line of lymphoblastoid human cells MT-2. The mechanism of anti-HIV action of LiCl(I)-I₂-α-dextrin-peptide complexes has been suggested.

Keywords: HIV-1 integrase; DFT-method; Molecular iodine complex

Introduction

HIV integrase inhibitors have a high therapeutic effect [1] for two reasons. First, integrase is one of the key participants in the virus replication cycle [2]. Second, integrase has no cellular equivalent and, hence, the suppression of its activity should not disturb normal cellular metabolism processes [3].

It is typical for such family of enzymes as HIV integrase to form very stable complexes with the DNA of a virus.

For integrase to be integrated it needs to bind both virus and cell DNA molecules at the same time. The integration proceeds in two stages and begins in the cytoplasm of HIV-infected cells where, upon completion of the reverse transcription of the virus DNA genome, integrase binds the virus DNA copy producing what is called the preintegration complex (PIC) which can be isolated from HIV-infected cells [4].

In the paper the authors compare the inhibiting activity of a series of antiretroviral agents in respect to the HIV-1 integrase and the in vitro-isolated PIC. The results of the study show that inhibitors active in respect to the integrase may not be active towards the PIC. The capability to inactivate the PIC was shown by three antiretroviral agents only [5].

Well-known are quite a number of iodine-polymer complexes that possess a broad spectrum of antimicrobial and antiviral action including human immunodeficiency virus (HIV).

Among them is Armenicum which is a drug to treat HIV infection [6-8]. Recently a new anti-infective drug with anti-HIV action has been patented (AID) [9].

The distinctive feature of those drugs is that their active substance includes not only the iodine-containing polymeric complex, but also potassium and lithium halogenides.

Under the influence of molecular iodine the whole of known microbial flora is killed, as it has antimicrobial and antiviral properties. However, all known iodine-containing drugs are characterized as highly toxic substances and therefore in fact are not used in medical practice for parenteral introduction. Both in Armenicum and in the new anti-infective drug molecular iodine adopts such an active form that when introduced orally it minimizes toxic effects in humans.

Armenicum is composed of the LiI₅-α-dextrin complex. The AID contains polypeptides along with the LiI₅-α-dextrin complex.

A model of the drug active complex of the AID and Armenicum was proposed based on X-ray data for LiI₅-α-dextrin complexes and the results of quantum-chemical ab initio RHF/3-21G** level calculations [10].

It has been shown that the drug active complex (AC) contains molecular iodine located inside the α-dextrin helix and coordinated by lithium halogenides and polypeptide. The electronic structure of I₂ in this complex differs from that in complexes with organic ligands or the free I₂ form. In the ACs under study the molecular iodine displays acceptor (donor) properties towards polypeptide (lithium halogenides) [10].

In this paper, using the quantum-chemical DFT / B3PW91 and UV spectroscopy data, we have shown that when LiCl(I)-I₂-α-dextrin-

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peptide complexes (LiCl(I)-I₂ DP) interact with the viral DNA, nucleotides displace peptide and form a complex with molecular iodine and lithium halogenides (LiCl(I)-I₂- α -dextrin-nucleotide). LiCl(I)-I₂- α -dextrin-nucleotide complex inhibits the active catalytic site of integrase inside the PIC.

The effect of antiviral action of LiCl(I)-I₂- α -dextrin-peptide complexes against HIV-1 has been established on a line of lymphoblastoid human cells MT-2. The mechanism of anti-HIV action of LiCl(I)-I₂- α -dextrin-peptide complexes has been suggested.

UV-spectroscopy research of interaction AC with nucleotide triplet AGA

We have suggested a mechanism of anti-HIV action of Armenicum and LiCl(I)-I₂ DP. In the active complex molecular iodine located inside the α -dextrin helix and coordinated by lithium halogenides and amide protein component appears to be hard-to-reach for interaction with bioorganic compounds.

Only bioorganic compounds whose donor activity is greater than that of amide can compete with it for producing complexes with I₂. The donor activity of nucleotides is greater than that of amides.

Having optimized the geometry of structures in which amide and nucleotide compete with each other for the formation of a complex with I₂ we have shown that the purine bases of adenosine and guanosine replace amide and form a complex with molecular iodine and lithium halogenides.

UV spectroscopy is one of the most widely used methods for studying the structure of DNA [11,12]. UV spectra are sensitive to the type of nucleotide base and to the formation of hydrogen or coordination bonds within the DNA. For that reason this method is often used to investigate the interaction of drugs with DNA nucleotides [13-15].

Using UV spectroscopy, we investigated the interaction of LiCl(I)-I₂ DP with the AGA nucleotide triplet.

Figure 1 shows the UV spectra of aqueous solutions of the AGA nucleotide triplet (system a) of the LiCl(I)-I₂ DP (system b) and the aqueous solution of the LiCl-I₂-DP complex and the AGA nucleotide triplet (system c).

Comparing the UV spectra of aqueous solutions of systems (a-c) shows that in system (c) the nucleotide triplet is observed to interact with the LiCl(I)-I₂ DP complex: ~ 287nm band observed in system (b) in the UV spectrum of system (c) disappears, while the band at ~ 256nm observed in system (a), becomes less intense and changes shape.

Using the DFT/B3PW91 method with the basis 6-31G ** for the atoms of C, N, O, H, Li, Cl and the midi basis for the atom of I with the complete optimization of geometry the structures of molecular iodine complexes with nucleotides adenosine (I) and guanosine (II) and LiCl (Figure 1) were obtained.

The TD-DFT/B3PW9 method was used to calculate the energies of electronic transitions in complexes I and II (Tables 1 and 2).

The splitting of the experimental band at ~ 200 nm can be described by electron transitions 206.51 nm, 210.20 nm and 218.63 nm in complex I. Transitions between π -orbitals of I₂ and π -orbitals of the nucleotide base contribute to these frequencies. The electronic transition due to the transfer of electron density from I₂ to lithium chlorine also contributes to the 210.20 nm frequency, while the transition between

the occupied orbitals of the coordination bond N-I and unoccupied orbitals I₂ contributes to the 218.63 nm frequency. Complex II also has the 215.09 nm transition between occupied and unoccupied orbitals I₂, which contributes to the band ~ 200nm.

Contributing to the 223nm band in complex I are the following two transitions: 226.57 nm and 234.55 nm, which may be referred to transitions between occupied and unoccupied orbitals of the nucleotide base of adenosine and to transitions between occupied and unoccupied orbitals of I₂. In complex II contributions to the said band are made by the 233.71 nm transition that corresponds to the transfer of electron density from the molecular iodine onto π -orbitals of the nucleotide base of guanosine.

Comparison of the quantum chemical calculations for electronic transitions for the structure modeling the interaction of LiCl-I₂-DP with the nucleotide triplet indicates that the DNA nucleotides can displace polypeptide and form stable complexes with molecular iodine and lithium halogenides. In such structures, molecular iodine binds both nucleotide triplet and lithium halogenides.

Thus, the conclusion made only on the basis of quantum-chemical calculations about the possibility of interaction of viral DNA nucleotides with LiCl(I)-I₂- α -dextrin complexes, that are part of drugs [6-10] is confirmed by UV spectroscopy and quantum chemical studies.

The mechanism of inhibition of the catalytic site of HIV-integrase

The structure of HIV-integrase can be divided into three domains: a short N-terminal domain, a catalytic domain, and a C-terminal domain. A model of the integrase structure in combination with the viral and cellular DNA was proposed in [16]. According to this model, it is the catalytic domain that binds the viral DNA, while all three domains bind the cellular DNA

The structure of the catalytic domain of integrase was determined by X-ray analysis. According to the data obtained, the catalytic domain in a crystal forms a spherical dimer with each monomer, shaped like a hemisphere. Three amino acid residues located close to one another in the tertiary structure of the catalytic domain form two active catalytic sites in each monomer, localized at the opposite end of the dimer. X-ray data clearly show a magnesium ion, coordinated by Asp64 Asp116 and two water molecules [17-18].

In [19] it is assumed that two Mg²⁺ are involved in the catalytic act, but due to the high conformational mobility of the catalytic fragment they may be bound only when they bind to the viral DNA. Using the method of molecular dynamics it is shown that the interaction with the viral DNA includes the integration of two Mg²⁺ in a stable binuclear complex, where the amino acid residue Glu152 simultaneously coordinates the two magnesium ions, whereas Asp64 and Asp116 interact with one magnesium ion only.

Recently X-ray diffraction data were obtained for the structure of integrase in combination with the viral DNA [20]. Based on these data, the authors suggest that the act of catalytical cleavage of the phosphoester bond involves two magnesium ions bound by amino acid residue Glu221, and each of the magnesium ions is coordinated by amino acid residues Asp128 and Asp185.

At its ends the viral DNA has direct repeats, consisting of hundreds of base pairs. At a distance of two nucleotides from the 3' -terminus of each DNA chain there is a conservative dinucleotide CA. Integrase as part of the PIC recognizes the nucleotide sequences located at the ends

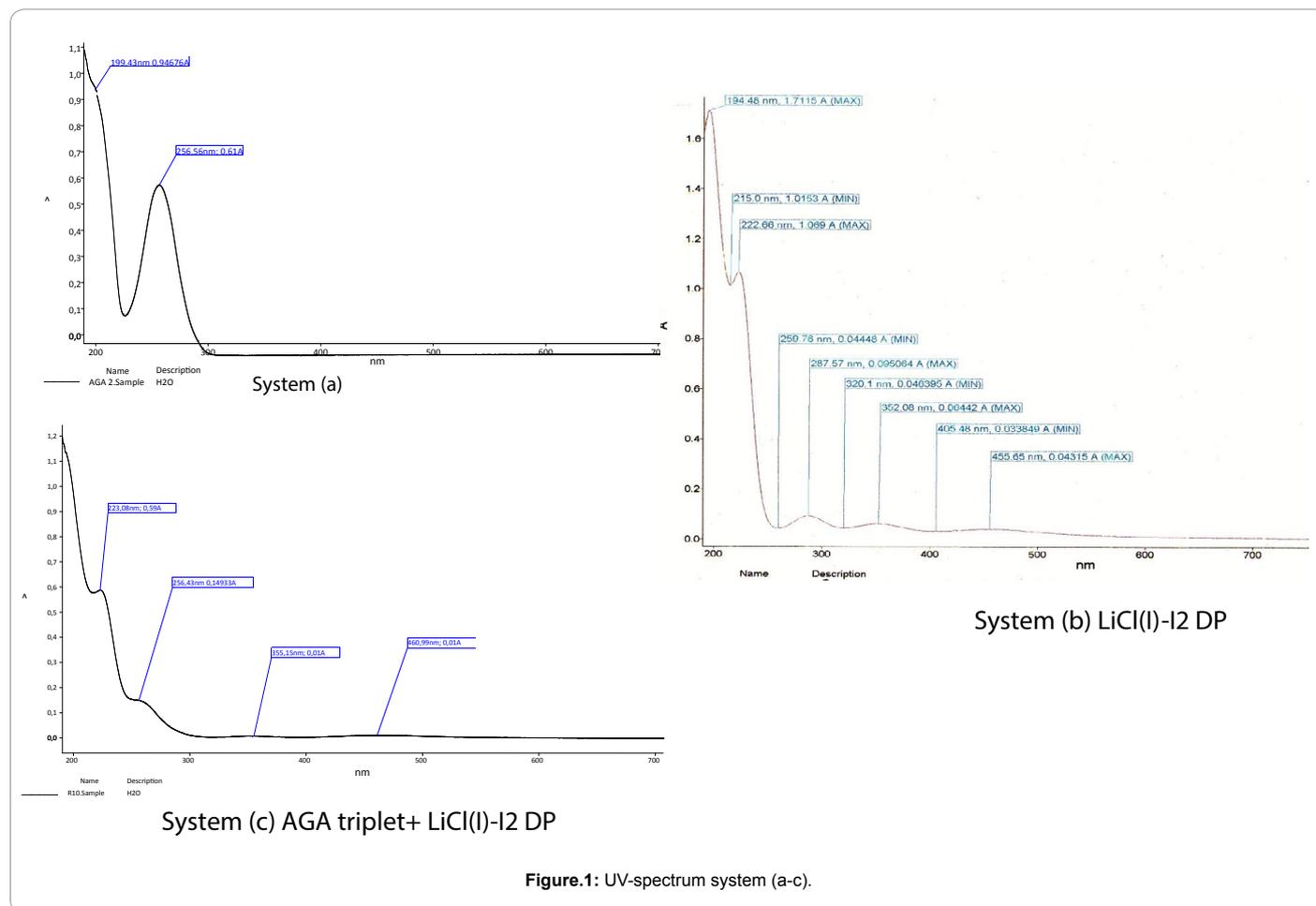


Figure 1: UV-spectrum system (a-c).

λ theor.	
206.51	${}^1A \rightarrow {}^1A (\pi(I_2) \rightarrow \pi (\text{nucleot.}))$
	${}^1A \rightarrow {}^1A (\pi (\text{nucleot.}) \rightarrow \pi (\text{nucleot.}))$
210.20	${}^1A \rightarrow {}^1A (\pi(I_2) \rightarrow \pi (\text{nucleot.}))$
	${}^1A \rightarrow {}^1A ((Cl-Li-I) \rightarrow \pi(I))$
218.63	${}^1A \rightarrow {}^1A (\pi (\text{nucleot.}) \rightarrow \pi(I_2))$
	${}^1A \rightarrow {}^1A ((N-I-I) \rightarrow \pi(I))$
226.57	${}^1A \rightarrow {}^1A (\pi (\text{nucleot.}) \rightarrow \pi(I_2))$
234.55	${}^1A \rightarrow {}^1A (\pi(I_2) \rightarrow \pi(I_2))$
	${}^1A \rightarrow {}^1A (\pi (\text{nucleot.}) \rightarrow \pi(I))$
244.34	${}^1A \rightarrow {}^1A (\pi (\text{nucleot.}) \rightarrow \pi (\text{nucleot.}))$

Table 1: Theoretical wavelengths (λ , nm) of electronic transitions in complex I.

of the viral DNA, binds to them and subsequently catalyzes the reaction of endonuclease cleavage of the viral DNA, which results in the removal of GT dinucleotide from the 3' end of each strand. The rupture of a phosphoester link in the phosphate group of the viral DNA is catalyzed by the active catalytic center of integrase.

In [21] the mechanism of the catalytic cleavage of the phosphoester bond was considered in the framework of molecular dynamics and DFT methods of quantum chemistry. The study begins with the construction of an initial transient state in which two magnesium ions coordinate the oxygen atom of the phosphoester group.

The authors do not consider the stable complex which is formed

λ theor.	
215.09	${}^1A \rightarrow {}^1A (\pi(I_2) \rightarrow \pi(I_2))$
233.71	${}^1A \rightarrow {}^1A (\pi(I_2) \rightarrow \pi (\text{nucleot.}))$
244.34	${}^1A \rightarrow {}^1A (\pi(I_2) \rightarrow \pi(I_2))$

Table 2: Theoretical wavelengths (λ , nm) of electronic transitions in complex II.

when the active catalytic site of the integrase interacts with the phosphate group of the viral DNA. However, this information is important when investigating the mechanisms of inhibition of an active integrase.

For the inhibition process against the activity of integrase to become possible, it is necessary that the binding energy of the drug in relation to the active site of integrase was comparable to or greater than its binding energy in relation to the phosphate group of the viral DNA.

In our calculations the structure of the binuclear active catalytic site of the integrase is represented by two magnesium ions bound by COOCH₃ group and each magnesium ion is coordinated by COOCH₃ group, while one of the ions is also coordinated by a water molecule.

In the calculated structures hydrocarbon and amide fragments of amino acid residues Asp64, Asp116, and Glu152 are replaced by a methyl group. This simplification of the structure of the amino acid residues is likely to be justified by the fact that the amide moiety is

separated from the carboxyl group by a number of methyl groups and therefore does not affect their donor activity.

In the structure of DNA the phosphate group is ionized at pH7.8, with a charge of -1. However, the metal ions (e.g. Na⁺, K⁺) present in the internuclear environment are complexed with the phosphate group and neutralize the negative charge.

In [22] by means of DFT-D using the potential BLYP-D it is shown that sodium ions as counterions make a significant contribution to the stability of the DNA double helix, because they reduce electrostatic repulsion of the phosphate groups and enhance the π - π stacking interaction of Watson-Krievskih pairs through the hydrophobic effect.

We considered two cases of neutralization of the charge of phosphate group by Na⁺ or H⁺, taking into consideration that ionization of the phosphate group is reversible. After ionization two oxygen atoms of the phosphate group become identical, and for that reason Na⁺ or H⁺, co-ordinates these two atoms.

Calculations have shown that in the most stable structures two magnesium ions displace Na⁺ or H⁺ to form coordination bonds with two oxygen atoms of the phosphate group. H⁺ is displaced from the coordination sphere of the phosphate group to form a covalent bond with one of the oxygen atoms of the carboxy group and forms a hydrogen bond with an oxygen atom of the other carboxy group. However, the displaced Na⁺ forms coordinate bonds with two oxygen atoms of the two carboxyl groups and with one of the oxygen atoms of the phosphate group

Assuming that the complex LiCl(I)-I₂- α -dextrin- nucleotide (adenosine, guanosine) may prevent the formation of a binuclear active catalytic site, we have considered two variants of interaction of magnesium ions with LiCl(I)-I₂- α -dextrin- nucleotide (adenosine, guanosine) complexes. In one case, the magnesium ion is coordinated by COOCH₃ group and a water molecule. In another case, the magnesium ion is coordinated by two COOCH₃ groups. In nucleoprotein complexes IVa,b-VIIa,b α -dextrin is replaced by a molecule of ethanol.

Figure 2 shows the binding energy of the active catalytic site of the integrase with the phosphate group (structures IIIa-b) and with complexes LiCl(I)-I₂- α -dextrin- nucleotide (adenosine, guanosine) (structure IVa, b-VIIa, b)

The binding energy ΔE_1 , ΔE_2 , ΔE_3 was calculated as follows below:

$$\Delta E_1 = E^{\text{tot}}(\text{IIIa}) - E^{\text{tot}}((\text{CH}_3)_2\text{PO}_4^- \text{Na}^+) - E^{\text{tot}}(\text{Mg}^{2+}(\text{COOCH}_3)_2) - E^{\text{tot}}(\text{H}_2\text{OMg}^{2+}(\text{COOCH}_3))$$

$$\Delta E_2 = E^{\text{tot}}(\text{IIIb}) - E^{\text{tot}}((\text{CH}_3)_2\text{PO}_4^- \text{H}^+) - E^{\text{tot}}(\text{Mg}^{2+}(\text{COOCH}_3)_2) - E^{\text{tot}}(\text{H}_2\text{OMg}^{2+}(\text{COOCH}_3))$$

$$\Delta E_3 = \Delta E_4 + \Delta E_5$$

$$\Delta E_4 = E(\text{IVa,b or VIa,b}) - E(\text{LiCl(I)-I}_2\text{'-dextrin-nucleotide(adenosine, guanosine)}) - E(\text{Mg}^{2+}(\text{COOCH}_3)_2)$$

$$\Delta E_5 = E(\text{Va,b or VIIa,b}) - E(\text{LiCl(I)-I}_2\text{'-dextrin-nucleotide(adenosine, guanosine)}) - E(\text{H}_2\text{OMg}^{2+}(\text{COOCH}_3))$$

instead $\Delta E_3 = E^{\text{tot}}(\text{IVa,b-VIIa,b}) - E^{\text{tot}}(\text{LiCl(I)-I}_2\text{'-dextrin-nucleotide(adenosine, guanosine)}) - E^{\text{tot}}(\text{Mg}^{2+}(\text{COOCH}_3)_2) - E^{\text{tot}}(\text{H}_2\text{OMg}^{2+}(\text{COOCH}_3))$. Our calculations show that in structures Va,b-VIIa,b the carboxy group replaces ion Cl(I) in lithium halogenide, and forms a binuclear complex with Li⁺ and Mg²⁺, while in structures IVa,b-VIa,b two carboxy groups bind Li⁺ and Mg²⁺ into the binuclear

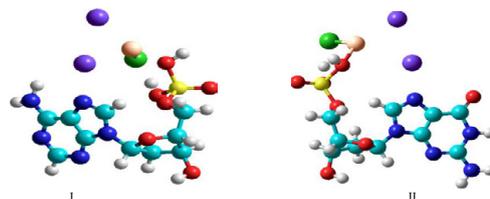


Figure 2: Complexes of molecular iodine with adenosine (I), guanosine (II) and lithium chloride. In the figure: blue balls-carbon atoms, dark blue balls – nitrogen atoms, red balls – oxygen atoms, violet balls – iodine atoms, orange balls – lithium atoms, green balls -chlorine atoms, yellow balls - phosphor atoms.

In the figure 3: blue balls-carbon atoms, dark blue balls – nitrogen atoms, red balls – oxygen atoms, violet balls – iodine atoms, green balls – magnesium atoms, orange balls-lithium atoms, yellow balls - phosphor atoms, black ball-halogen atom.

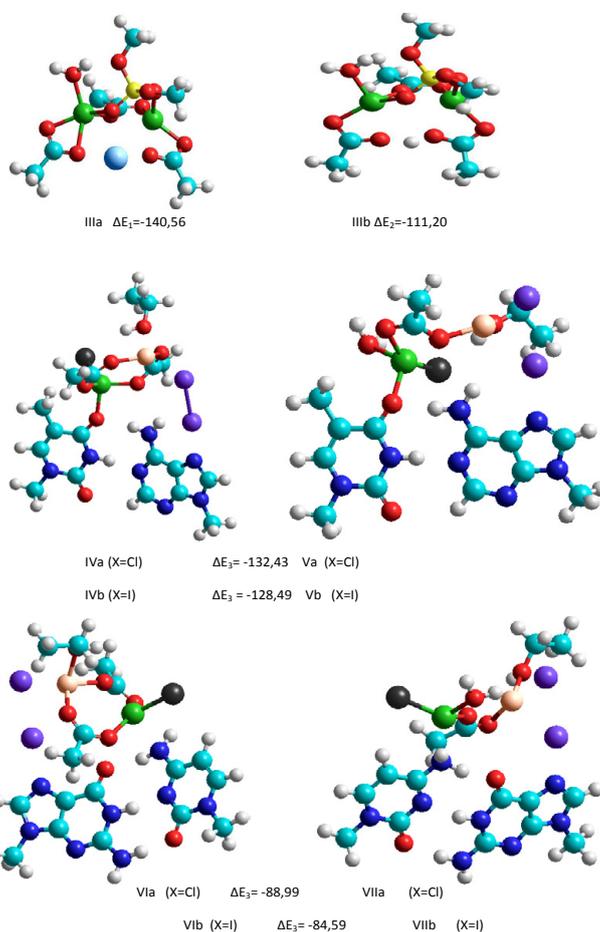


Figure 3: shows the binding energy of the active catalytic site of the integrase with the phosphate group (structures IIIa-b) and with complexes LiCl(I)-I₂- α -dextrin- nucleotide (adenosine, guanosine) (structure IVa, b-VIIa, b).

complex. Also Mg²⁺ forms a coordination bond with Cl⁻ and the oxygen atom of the carbonyl group of thymine in structures IVa, b-Va, b or the nitrogen of the amino group of cytosine in structures VIa,b-VIIa,b.

As can be seen in Figure 3 it is only LiCl(I)-I₂- α -dextrin-adenosine complexes that can compete with the phosphate group for binding the catalytic active center, because in this case ΔE_3 lies between ΔE_1 and

ΔE_2 .

The great difference in stability of IVa,b-Va,b and VIa,b-VIIa,b structures is due to low donor activity of the nitrogen atom in the amino group of cytosine as compared to that of the oxygen atom of the carbonyl group of thymine.

Thus, LiCl(I)-I₂- α -dextrin complexes, that are part of the active centre of drugs [6-10] inhibit the catalytic activity of the active catalytic site of integrase within the PIC. Lithium ion becomes a trap for the Mg²⁺(COOCH₃)₂ and H₂OMg (COOCH₃)⁻ complexes, that form the structure of the active catalytic site of integrase. In the presence of LiCl(I)-I₂- α -dextrin- complexes magnesium ions are coordinated by the carbonyl oxygen atom of thymine rather than by the atoms of oxygen in the phosphate group. The conditions that ensure the occurrence of catalytic cleavage of the phosphoester bond are disturbed.

In IVa,b-Va,b nucleoprotein complexes the spatial structure of Watson - Crick pairs gets deformed, the planes of adenosine and thymine molecule are located at an angle of ~15°, while the distance between oxygen and hydrogen atoms linked by intermolecular hydrogen bond becomes equal to ~2,03Å (hydrogen bond O --- H breaks.), while that for hydrogen and nitrogen atoms becomes equal to ~1,80Å.

Experimental Research

Materials and methods

Tested compound – AID [9]: The compounds under study were first dissolved in absolute ethanol to 1:1000 dilution and after that further diluted in cell growth medium without fetal serum. All solutions were prepared *ex tempore*.

Cell line and virus: MT-2 cell line used routinely as a target for study of acute HIV-1 infection. H9/HTLV III B cell line (a gift from Dr. R. Gallo (Natl. Inst. Of Health, USA) was chronically infected and produced HIV-1_{LAI} strain. As a source of HIV-1, the supernatant of H9/HTLV III B cell line was used. The supernatants were collected and centrifuged to remove the cells, and virus stocks were prepared with known p24 antigen content, RT activity and infectivity.

MT-2 cells were infected with HIV-1 (multiplicity of infection ≥ 1) in suspension (37°C/5% CO₂) and then grown parallelly with uninfected cells for 72 hours.

Anti-HIV assays

The infectivity was measured on MT-2 cells by microtiter infection assay using MTT test. Briefly, the experimental procedure under conditions of acute infection was performed in 96-well microplates previously treated for an hour with 50 μ g/ml poly-L-lysine (PLL) in volume 100 μ l in the following sequence:

- 1/ 100 μ l MT-2 cells (5 – 7 $\times 10^4$ cells) earlier infected with HIV-1 (1 hour, 37°C, 5% CO₂) – i.e. virus control or uninfected - i.e. cell control;
- 2/ 50 μ l sample of substance to be tested starting from 4 x MNC (because this volume is 1/4 of the whole volume in the well) and 2 or more 10-fold dilutions in growth medium;
- 3/ 50 μ l growth medium.

So, each plate contained 6 - 8 cell controls (no virus, no substance) for all experiments, rows of 6 - 8 viral controls (plus virus, no substance) and rows of 6 - 8 experimental wells (plus virus and plus

different dilutions of the substance). The values of optical density at 540 nm (A540) were averaged for each row. The mean values of experimental and control rows were compared and the percentage of cells protected under the appropriate concentration of the substance studied was plotted against the concentration of the substance.

The cells were incubated for 72 hours. The tissue culture fluid (supernatants) from each well was collected and removed in appropriate (mirror-like) non-sterile 96-well plate and stored in 4°C until the results of A540 reading were ready.

Detection of Reverse Transcriptase (RT) activity

Endogenous RT activity of supernatants of HIV-1 infected MT-2 cells treated/untreated with the substances was tested by HS-Lenti Kit-RT assay (Cavidi, Sweden). The kit contained recombinant RT (rRT) as a standard which made possible the quantitation of the RT in the samples. The method is non-radioactive and colorimetrically detects the product of reverse transcription at 405 nm (A405). The activity was expressed as % from the viral control.

Detection of the effect of the substances on recombinant Reverse Transcriptase (rRT) activity

A possible direct action of the substances having shown anti-HIV-1 effect on MT-2 infected cells measured by RT assay in supernatants was further studied using rRT standard provided by Cavidi kit. The reaction mixture for the assay was prepared with the active substance (in the appropriate dilutions) or without them and the reverse transcription was carried out with the standard rRT. A405 was then measured for the samples where the active substance has been added in different solutions and compared to that of the control (where only incubation mixture without any substance was added). The rRT activity was expressed as % compared to the control positive reaction.

Results and Discussion

Each experiment has been done in triplicates; each run was carried out with 6 – 8 parallels. Only parallels differing in less than 10% in A540 were collected for further RT assay.

¹MNC – was evaluated as maximal nontoxic concentration of the substance which altered neither the morphology nor the survival rate of MT-2 cells.

²mean values of six to eight parallels per experiment with standard deviation $\leq 10\%$ in MTT test; each experiment run in triplicate. % cell survival at different inhibitor concentrations.

³from duplicate experiments. RT activity is the ratio between the RT in supernatants of inhibitor-treated HIV-1 infected MT-2 cells and that in untreated HIV-1 infected MT-2 cells (virus control, 100%). % inhibition is 100% - % RT activity (not shown in the Table 3).

⁴from duplicate experiments. rRT activity was measured in incubation mixture without addition of inhibitor (100%) and compared to the same activity in incubation mixture where inhibitor was added in appropriate concentration. % inhibition is 100% - rRT activity (not shown).

NA – not applicable

Table 3 shows a remarkable inhibition of RT activity in supernatants of HIV-1 infected MT-2 cells treated with AID substances in 2x dilutions. At the same time no inhibition is seen when the compounds are added directly to the incubation mixture to allow reverse transcription with a recombinant RT – i.e. the inhibition observed in tissue culture is not due to direct action on the RT enzyme.

Substance	Compound Concentration (dilution)	Infection assay MTT-A540 (% cell survival ²)	Supernatant RT activity A405 ³ (Control) % inhibition	Incub. mixture rRT activity A405 ⁴ (Control) % inhibition
AID	1:5000 (MNC ¹)	1,840 (92,9)	0,713 (2,703) 73,7	2,096 (2,258) 0
	1:10000	1,902 (96,0)	0,744 (2,703) 72,5	2,179 (2,258) 0
	1:20000	1,946 (98,2)	0,807(2,703) 70,2	2,165 (2,258) 0
Cell control	0	1,980 (100)	0,231 0	NA
Virus control	0	1,315	2,703 100	NA

Table 3: AID inhibition of HIV-1 replication in MT-2 cells expressed in MTT infection assay and by RT and rRT activity.

These observations mean that both compound LiCl(I)-I₂ DP inhibit HIV-1 replication. This is clearly demonstrated by the inhibition of RT activity in the supernatants of MT-2 infected cells. The target and mechanism of action of compound should be further elucidated by looking for another viral targets – i.e. integrase and/or protease .

Conclusion

The water-nucleotide AGA triplet - LiCl(I)-I₂-α-dextrin-peptide system was investigated using UV spectroscopy. The good agreement between the experimental UV-frequencies of the system with the theoretical frequencies for LiCl(I)-I₂-α-dextrin-nucleotide complexes indicates that when interacting with LiCl(I)-I₂-α-dextrin complexes AGA triplet nucleotides displace peptide and form a new complex with iodine and lithium halides.

Using the quantum-chemical DFT it was shown that the LiCl(I)-I₂-α-dextrin-nucleotide complexes inhibit the activity of the catalytic site of integrase inside the PIC. They disrupt the conditions ensuring the occurrence of catalytical cleavage of the phosphoester bond in the phosphate group of the viral DNA by the active catalytic site of integrase. They form a nucleoprotein complex consisting of the nucleotides of the viral DNA, LiCl(I)-I₂-α-dextrin complexes and active components of the catalytic site of integrase. In these complexes magnesium ions are coordinated by the carbonyl oxygen atom of thymine rather than by the oxygen atoms of the phosphate group.

The effect of antiviral action of LiCl(I)-I₂-α-dextrin-peptide complexes against HIV-1 has been established on a line of lymphoblastoid human cells MT-2. The mechanism of anti-HIV action of LiCl(I)-I₂-α-dextrin-peptide complexes has been suggested.

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