# Ionic strength effect on violamycin B1 – DNA interactions

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*We studied the influence of the ionic strength on the interaction between violamycin V B1 and DNA in aqueous solutions. One evidenced two types of bindings one in the stacked complex on DNA surface and the other in the intercalated complex between DNA base pairs. In both cases was evidenced the competition between V B1 molecules and $Na^+$ ions to occupy the binding sites. The binding parameters of both complexes were determined and compared to those obtained in phosphate buffer. Our study showed that over a value of 0,02M the binding parameters don't modify substantially.*

*Keywords: violamycin, DNA, ionic strength, binding, parameters*

The binding interaction of the anthracycline antibiotics to DNA, in phosphate buffer, has been extensively studied [1-5]. The V B1 is an anthracycline antibiotic with the highest cardiotoxicity. It was previously investigated its interaction with the synthetic single stranded [6] and synthetic double stranded nucleic acids [7-9] as well as with DNA [10-12], in phosphate buffer aqueous solutions. It was showed that V B1 interacts with DNA by nonspecific and specific interactions. The first type of interaction consists mainly in electrostatic attraction between the negative phosphate groups on the DNA helix and the V B1 glycosidic moieties with positive charges on nitrogen from V B1. In addition, cooperativity and hydrophobic forces contribute also to this type of surface binding.

The specific interactions are due to the intercalation of the V B1 aglycone.

Being known that the living cell has 0,17 M ionic strength and the anticancer drugs have to act under such condition, were developed studies [13-16] regarding the effect of ionic strength upon the binding parameters of different ligands to DNA.

This paper refers to the influence of ionic strength upon binding parameters of V B1 to DNA in both types of complexes.

## Experimetal part

*Materials and methods*

Violamycin IMET-JA 6844 was prepared for the first time from *Streptomyces violaceus* [17] and offered us by Dr. D. G. Strauss. Aqueous solutions of V BI were prepared in the presence of NaCl of 0.000 M, 0.005M, 0.01M and 0.02M and kept in the dark before measurements to avoid its photobleaching. V B1 concentration was determined by measuring the absorbance at $\lambda$=500nm, the molar absorption coefficient being known [18] namely $\varepsilon_{500}$=10250 $M^{-1}cm^{-1}$.

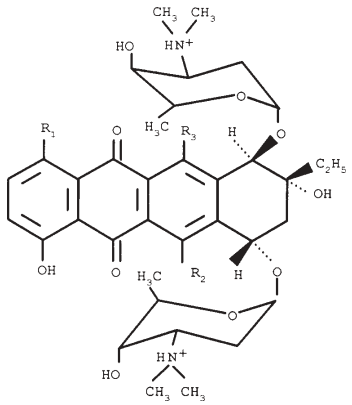
DNA calf thymus from SIGMA was used as sodium salt. DNA solutions were prepared in the aqueous solutions with the same ionic strength as for V BI. Its concentrations were determined by measuring the absorbance at: $\lambda$=258 nm ($\varepsilon$=6600 $M^{-1}cm^{-1}$) [19].

The absorption spectra were measured at an Unicam $\alpha$ Helios spectrophotometer and the fluorescence spectra at an Aminco Bowman spectrofluorimeter. For the determination of binding parameters we carried out the titration of V BI with DNA into the spectral cell. The addition of the DNA into V B1 solution in the measure cell was accompanied each time by the addition of an equal volume of acid in the reference cell to prevent matrix differences between the two cells. The experiments were carried out at $25^0$C.

## Results and discussions

Violamycin B1 is an equimolecular mixture of three isomers and has the following structure:

An antracyclinic skeleton, the chromophore of the molecule, at which are attached two glycosidic residues, each one with a positive charge on the nitrogen.



$R_1$= H; $R_2$= OH; $R_3$= OH
$R_1$= OH; $R_2$= OH; $R_3$= OH
$R_1$= OH; $R_2$= H; $R_3$= OH

We carried out the titration of the V B1 ($3.00 \times 10^{-5}$M) with DNA ($2.05 \times 10^{-3}$MP) exemplified for the ionic strength I=0.005M in figure 1 and found similar behaviour as the titration of the same system in phosphate buffer [10].

Figure 1a reveals a pronounced hypochromic effect with the appearance of two isosbestic points at $\lambda$=570 nm and $\lambda$=620 nm which attests the presence of the equilibrium between free and bound V B1 in the surface complex.

If we add continuously DNA in the spectrophotometric cell, increasing the value of **p** (the ratio between DNA and V B1 concentrations) we remark also a pronounced shifting of the absorption maximum towards the long wavelengths accompanied by its splitting which reveals the presence of a new equilibrium between free and bound V B1 in the intercalated complex.

In figure 1b it can be observed the decrease of the fluorescence intensity and the shifting of its emission maximum towards the short wavelengths due to the formation of these complexes.

Figure 2 presents our experimental data from the both spectral titration, by plotting $\gamma(A/A_o$ in absorption and $I/I_0$

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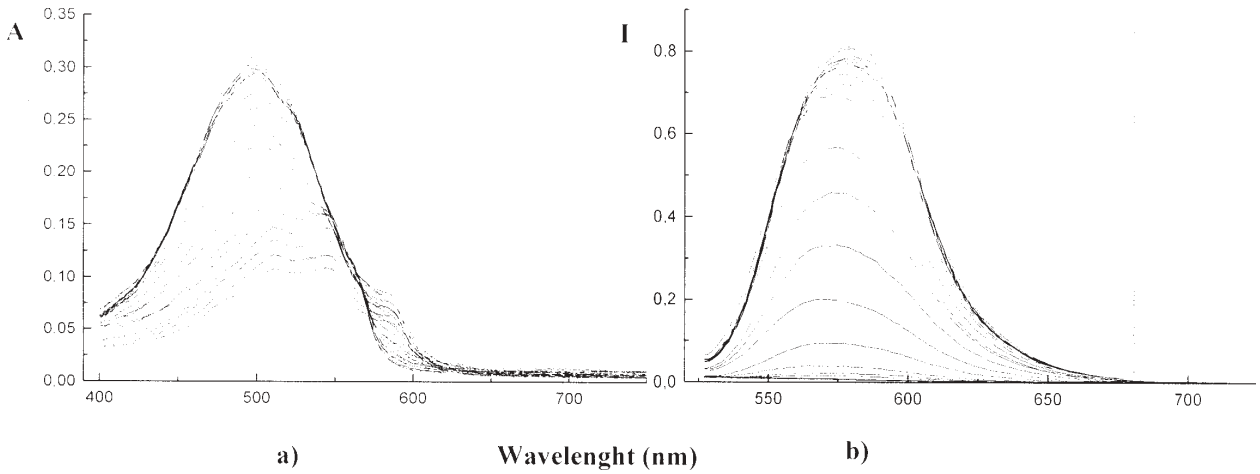


Fig. 1 The spectrum evolution in a) absorption and b) fluorescence emission during the titration of V B1 with DNA at 0.005M ionic strength

in emission) versus **p**. The two curves have similar shape, namely a deep decrease at the beginning of the titration (low values of **p**), followed by a moderate one with the tendency to reach a plateau at high p values. However there is also an important difference: whereas the fluorescence emission is practically quenched at the finish of the titration, the absorbance has still measurable values indicating that the formed complexes absorb in the same spectral domain with the free violamycin.

For this reason we preferred the spectrofluorimetric data to calculate the binding parameters preventing the overlapping of the absorbance measurements.

By using the Schwarz model [20-23] we calculated the binding parameters al low values of **p**, by plotting $\gamma^*$ (the total fraction of free V B1 in its monomeric and possible dimeric form) versus **p**, as figure 3 shows.

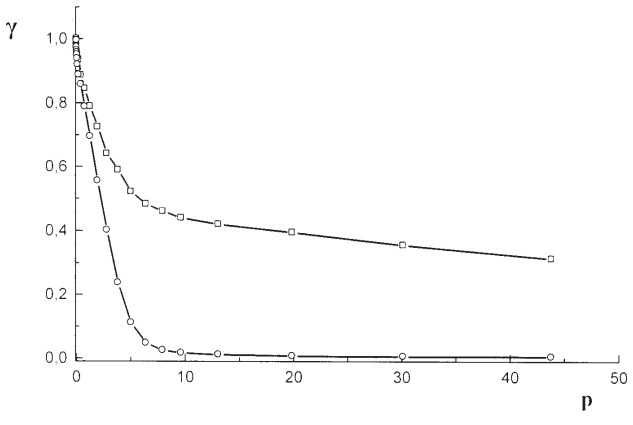


Fig. 2. $\gamma$ versus **p** ($A/A_0$ (%) and $I/I_0$ (o)

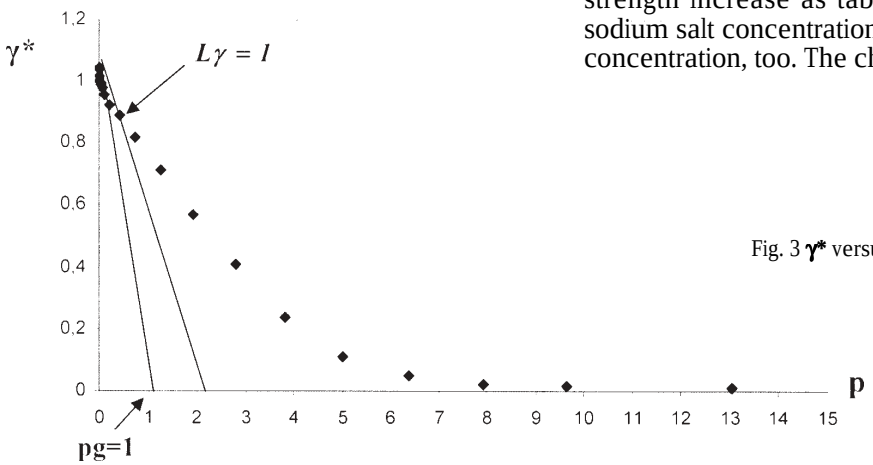


Fig. 3 $\gamma^*$ versus **p** (Schwarz method)

$\gamma^* = \gamma(1+2K_D c^o_{VB}\gamma)$, $K_D$ being the dimerization constant of free monomeric violamycin in solution, with a value of 730 $M^{-1}$ determined by us in a previous paper [24] and $\gamma$ the fraction of free monomeric V B1.

By extrapolating this linear part from the beginning of the titration, it intersects the abscissa in a point for which **pg**=1. The parameter **g** is the number of the binding sites occupied by a single V B1 molecule, in the nucleation process. Then, V B1 molecules superposed on the first one forming aggregates of different lengths. The formed stacked complex is characterized by a stability constant $K_{st}$. For the calculation of this parameter it was drawn a straight line with the slope –g/2 which intercept the binding curve at a point for which $\gamma L=1$, L being the binding strength of V BI in the stacked complex. It depends on the initial concentration of V BI as follows: $L=K_{st} c^o_{VBI}$.

Our experimental data concerning the binding parameters of V B1 stacked complex in water and at three different ionic strengths are summarized in table I [25].

Taking into account the significance of L and V B1 concentration practically constant, it was expected the value of L=1.1 (the fourth column of the table 1).

For all samples containing NaCl we determined a **g** value of 0.5, similar with that obtained in phosphate buffer [10] (I=0.02M) meaning that one V B1 molecule occupies 2 binding sites. We remark that **g** is in this case smaller that in pure water. Keeping in mind that **g** represents the number of binding sites per monomeric unit, we have to conclude that in the presence of salt the binding sites of DNA are partially occupied by $Na^+$ ions, strongly electrostatically attracted by $PO_4$ groups than V B1 molecules.

The $K_{st}$ values present a slightly decrease with ionic strength increase as table I shows. The increasing of sodium salt concentrations determines a higher chloride concentration, too. The chloride ions are attracted by the

**Table 1**

THE BINDING PARAMETERS OF V B1 STACKED COMPLEX AT DIFFERENT IONIC STRENGTHS

| I, M | $c^{0}_{VB}$ x $10^{-5}$, M | $c_{DNA}$ x $10^{-3}$, MP | L | g | $K_{st}$ x $10^{-4}$, $M^{-1}$ | q | $K_n=K_{st}/q$ |
|---|---|---|---|---|---|---|---|
| 0.000 | 3.50 | 2.24 | 1.4 | 2.4 | 3.93 | 6 | 7017 |
| 0.005 | 3.00 | 2.05 | 1.1 | 0.5 | 3.70 | 19 | 1947 |
| 0.01 | 3.14 | 2.42 | 1.1 | 0.5 | 3.53 | 51 | 692 |
| 0.02 | 3.44 | 2.06 | 1.1 | 0.4 | 3.06 | 48 | 637 |

positive V B1 and form a negative shell so that between V B1 molecules stacked on DNA surface the repulsion forces are diminished. This is the reason of highly increase of cooperativity parameter (**q**) with the ionic strength. It can be noticed that over the value I=0.01M this parameter tend to be constant at a value of 50.

In the last column we calculated the nucleation equilibrium constant $K_n$ at the monomeric binding of V B1 on DNA surface. This constant has the highest value in water when $PO_4^-$ sites are free, not occupied by sodium ions, so the nucleation process may starts at any site.

We use the $K_{st}$ values to calculate intrinsic binding constant of sodium ions to DNA chain $K_i$ and intrinsic binding constant for V B1 to DNA $K_0$, accordingly to the ecuation (1):

$$K_{st} = \frac{K_0}{1 + K_i a_{NaCl}} \qquad (1)$$

where $a_{NaCl}$ is the medium activity $a_{\pm}$.

By plotting the reciprocal equation (1) as figure 4 shows, we obtained a straight line which intersect the ordinate at $1/K_0$ and has the slope $K_i/K_0$.

We calculated $K_0 = 3.33 \cdot 10^4$ $M^{-1}$ and $K_i = 13.8$ $M^{-1}$. $K_0$ represents practically the value of $K_{st}$ at a ionic strength I=0 that is the value of $K_{st}$ in distillated water. Indeed, the found value $3.33 \cdot 10^4$ $M^{-1}$ is in good agreement with the value $3.53 \cdot 10^4$ $M^{-1}$ determined by us in distilated water.

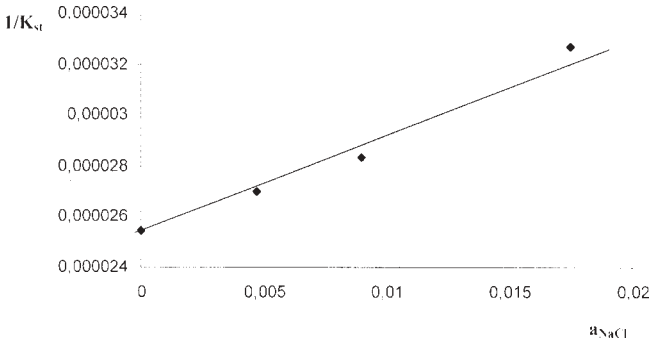


Fig. 4 $1/K_{st}$ variation versus $a_{NaCl}$

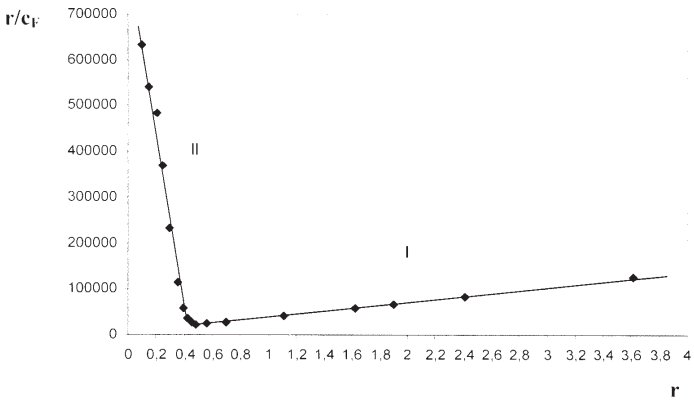


Fig. 5 Scatchard plotting $\mathbf{r/c_F}$ vs **r**

Schwarz [21] determined a value of $K_i = 170$ $M^{-1}$ for $Na^+$ binding to linear polianions (e.g. poliacrilic acid) and a value of $K_i = 199$ $M^{-1}$ for polycitidilic acid was determined [26]. We attribute our very low $K_i$ value (13.8 $M^{-1}$) to the competition between V B1 and $Na^+$ to bind $PO_4^-$ groups. Indeed, if we look at the Table I where the last column gives the value of nucleation equilibrium constant $K_n$, we observe the systematical decreasing of $K_{st}$ with $Na^+$ increasing.

If one continues the titration starting from **p**=1.2 when the formation of stacked complex is practically finished, it forms the intercalated complex by the penetration of the ligand between the DNA base pairs. We applied Scatchard method [27] to calculate its binding parameters by plotting the equation (2) where **r** is the ratio between the bound V B1 and DNA concentrations showed in figure 5.

$$r/c_F = K_{int}(B_{cap} - r) \qquad (2)$$

There are two linear segments; the first one with a positive slope attributed to the stacked complex formation discussed before, for **r**>0.5 (**p**>1.2).

The segment II ranging 0.15<**r**<0.5 (1.2<**p**<13) refers to the intercalated complex formation, characterized by a negative slope according to Scatchard equation (2) from which we calculated this stability constant $K_{int}$. The negative slope shows that the complex formation seems to be a noncooperative process.

We studied also, the influence of the ionic strength on the equilibrium constant of this complex. In the Table II are presented our results.

The ionic strength increasing, as Table II shows, leads to the decrease of the equilibrium constant of the intercalated complex $K_{int}$ and the **n** increasing. The last column of **n** suggests that V B1, which intercalates at each 2-3 base pairs in water, begin to increase with $Na^+$ concentration up to value of 5. This behaviour is due to the fact that $Na^+$ occupies the majority of the binding sites and V B1 finds free sites only after 5 base pairs.

We showed in a previous paper [10] that the intercalation of V B1 is accompanied by the electron transfer from the base to the ligand in its excited singlet

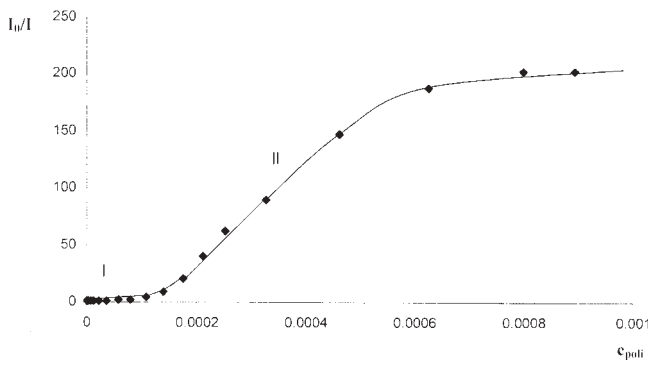


Fig. 6. Stern Volmer equation plotting

| I, M | $c^{II}_{VB}$, M | $c_{DNA}$, MP | $K_{int}$ x $10^{-6}$, $M^{-1}$ | $B_{ap}$ | n = 1/$B_{ap}$ |
|---|---|---|---|---|---|
| 0.000 | 3.50 x $10^{-5}$ | 2.24 x $10^{-3}$ | 9.55 | 0.4 | 2.5 |
| 0.005 | 3.00 x $10^{-5}$ | 2.05 x $10^{-3}$ | 2.22 | 0.5 | 2 |
| 0.01 | 3.14 x $10^{-5}$ | 2.42 x $10^{-3}$ | 1.83 | 0.3 | 3.3 |
| 0.02 | 3.44 x $10^{-5}$ | 2.06 x $10^{-3}$ | 1.62 | 0.2 | 5 |

**Table 2**
THE BINDING PARAMETERS OF THE INTERCALATED COMPLEX AT DIFFERENT IONIC STRENGTHS

| I, M | $c^{II}_{VB}$, M | $c_{DNA}$, MP | $k^{II}_{SV}$ x $10^{-5}$, $M^{-1}$ | $k^{II}_{q}$ x $10^{-13}$, $Ms^{-1}$ |
|---|---|---|---|---|
| 0.000 | 3.50 x $10^{-5}$ | 2.24 x $10^{-3}$ | 8.36 | 20.30 |
| 0.005 | 3.00 x $10^{-5}$ | 2.05 x $10^{-3}$ | 3.84 | 9.36 |
| 0.01 | 3.14 x $10^{-5}$ | 2.42 x $10^{-3}$ | 2.63 | 6.42 |
| 0.02 | 3.44 x $10^{-5}$ | 2.06 x $10^{-3}$ | 2.28 | 5.56 |

**Table 3**
$k_{SV}$ AND $k_q$ OBTAINED AT THE INTERCALATION FOR DIFFERENT IONIC STRENGTHS

state. As a support for this affirmation comes our quenching fluorescence experiment showed in figure 6.

Because during the titration, due to the formation of the two complexes takes place the quenching of fluorescence we considered interesting to prove if Stern Volmer equation (3) is obeyed. In Figure 6 we exemplified this equation for ionic strength 0.005 M.

$$I/I_0 = 1 + k_{SV} [c_{DNA}] \quad (3)$$

One observes again the two distinct linear segments of the stacked and intercalated complex formation. Then the curve reaches a plateau because the free violamycin was completely consumed so that there is no emissive species in the solution.

From the segment II which refers to the intercalated complex we calculated the Stern Volmer constant $k_{SV}$. Because we determined experimentally also the average life time of violamycin in its singlet excited state of 4.1 . $10^{-9}$ s, we were able to calculate the quenching rate constant $k_q = k_{SV}/\tau_{V\,B1}$ for all the ionic strengths used in our experiments. We presented the obtained results in table 3.

The rate constant for fluorescence quenching ($k_q$) with order of magnitude $10^{-13}$ $Ms^{-1}$ shows a very fast process according to the electron transfer. One observes that the rate constant $k_q$ which represents the electron transfer from the base to V B1 decrease with the increasing of ionic strength. This result shows that $Na^+$ moves faster than V B1 molecules to enter between the base pairs so that the electron of the base is transferred rapidly to $Na^+$ ions rather than to V B1. Indeed, the data obtained put into evidence again the competition between V B1 molecules and $Na^+$ ions in accepting the electron transferred from the bases.

At higher ionic strength that 0.02M all the studied binding parameters modify slower with the tendency to reach a constant value. This case is present in the living cell with the ionic strength of about 0.17M.

## Conclusions

The formation of the surface and the intercalation complexes characterized by the corresponding binding parameters of V BI to DNA are strongly influenced at concentration of salt lower than 0.02 M.

Regarding the surface complex formation we showed that the nucleation equilibrium constant $K_n$ (the monomeric binding of V B1 to phosphate groups along DNA chain) is strongly affected by the presence of sodium ions which bind electrostatically faster to DNA surface. In exchange, the stacking complex is much less affected by the ionic strength.

Concerning the intercalated complex, the increasing of the ionic strength leads to a wicker binding of V B1 and only after few base pairs. It was evidenced the electron transfer from the bases to V B1 in competition with the transfer to $Na^+$ ions, in the intercalated complex. Therefore value of the rate constant $k_q$ decreases with the ionic strength increasing.

Such studies are necessary to understand the behaviour of anticancer drug in the living cell characterized by high enough ionic strength.

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