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Letter

Dimerization kinetics of violamycin BI anthracycline — the influence of ionic strength

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Abstract

Violamycin BI is an anthracycline derivative with two sugars hanging on, each of them carries one positive charge. It dimerizes under conditions, which depend on the concentration of the antibiotic, pH and the ionic strength of the solution. By keeping a constant pH in a phosphate-EDTA buffer, the rate constants of violamycin BI dimerization were determined at various ionic strengths by temperature jump method. The dimerization constant K_d, resulting from the ratio of these rate constants, confirmed the values obtained spectrophotometrically in this study or elsewhere. The influence of ionic strength (0.02–0.2 M) on the rate constant values suggested to us some speculations on the reaction mechanism of the dimerization, in which, the specific mutual orientation of the monomers in the encounter, and perhaps a specific conformation of their side groups is required before a stabilizing action of the binding forces sets in. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Anthracycline antibiotics (adriamycin, iremycin, carminomycin, etc.) are known to form aggregates in solution [1–4]. These anthracyclines have a single positive charge and self-associate like the cationic dyes. Violamycin BI is also an anthracycline antibiotic but with two positive charges. Löber et al. [5] pointed out that it presents self-association in solution too, however, they did not study it.

A quantitative study on its dimerization was first carried out in a phosphate–EDTA buffer containing 0.2 M NaCl [6]. In this medium at 20.5°C, the dimerization constant K_d, determined

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spectrophotometrically, is 9355 ± 300 M^{-1}. Later we determined this equilibrium constant in the same buffer but in the absence of NaCl [7] and found a value of 732 ± 50 M^{-1}. As expected the high ionic strength favors the dimer formation of violamycin molecules.

2. Materials and methods

Violamycin BI was provided by Dr D.G. Strauss, for which, we are indebted. The solutions were always freshly prepared in the phosphate–EDTA buffer with an ionic strength adjusted by the addition of NaCl. All chemicals were analytical grade reagents (Merck).

The concentrations of VBI were determined by measuring the absorption at $\lambda = 500$ nm using a molar absorption coefficient $\varepsilon_{500} = 10.250$ M^{-1} cm^{-1} [6]. The absorbance measurement necessary to determine the dimerization constant K_d was carried out at a Unicam UV/Vis Helyos α spectrophotometer.

For the kinetic experiments we used a Relaxation Temperature Jump Spectrometer, Studien Messanlagen, Göttingen. The relaxation curves were obtained at 20.5°C and at $\lambda = 546$ nm, emitted by a Hg lamp.

3. Results

In the present paper we extended our study to other ionic strengths in the range 0.02 (the natural ionic strength of the buffer) to 0.22 M. We used the temperature jump method to obtain information also on the kinetics of this equilibrium:

$$2M \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} D \quad (1)$$

characterized by the rate constants k_{12} and k_{21} and the equilibrium constant $K_d = k_{12}/k_{21}$. For this purpose we started with the dimerization in the absence of NaCl to compare the value obtained from these kinetic experiments with those of the spectrophotometric study [7]. The relaxation curves show a simple relaxation behavior characterized by a single relaxation time. The absorbance increasing with the temperature is supported by the dissociation of the dimer (the extinction coefficient of the chromophore is higher in the monomeric form). The dimerization process must, therefore, be exothermic with a negative standard dimerization enthalpy, which we already showed [6]. Our results obtained from the temperature jump experiments are summarized in Table 1.

The characteristic relaxation Eq. (2) for a dimerization process is obeyed as shown in Fig. 1.

$$\tau^{-2} = k_{21}^2 + 8k_{12}k_{21}c^o_{VBI} \quad (2)$$

in which τ is the relaxation time, c^o_{VBI} the initial (total) concentration and k_{12}, k_{21} the rate constants involved in Eq. (1).

The values $k_{21} = 2.00 \times 10^4$ s^{-1} and $k_{12} = 1.50 \times 10^7$ $M^{-1}s^{-1}$ are obtained from the intercept and slope, respectively. Their ratio yields to $K_d = 750 \pm 80$ M^{-1} in good agreement with the value 732 M^{-1} obtained from spectrophotometric experiments [7]. We proceed in the same manner for each investigated ionic strength, namely 0.045, 0.070 and 0.12 M. The corresponding data for the ionic strength 0.22 M are known from [6]. Table 2 summarizes our results.

Table 1
The relaxation times of the dimerization process at different VBI initial concentrations, in phosphate–EDTA buffer at 20.5°C

$c^o_{VBI} \times 10^4$ M	$\tau^{-1} \times 10^4$ s^{-1}	$\tau^{-2} \times 10^8$ s^{-2}
4.50	3.95	15.64
3.00	3.24	10.50
2.50	3.29	10.82
1.00	2.65	7.02
0.47	2.16	4.65

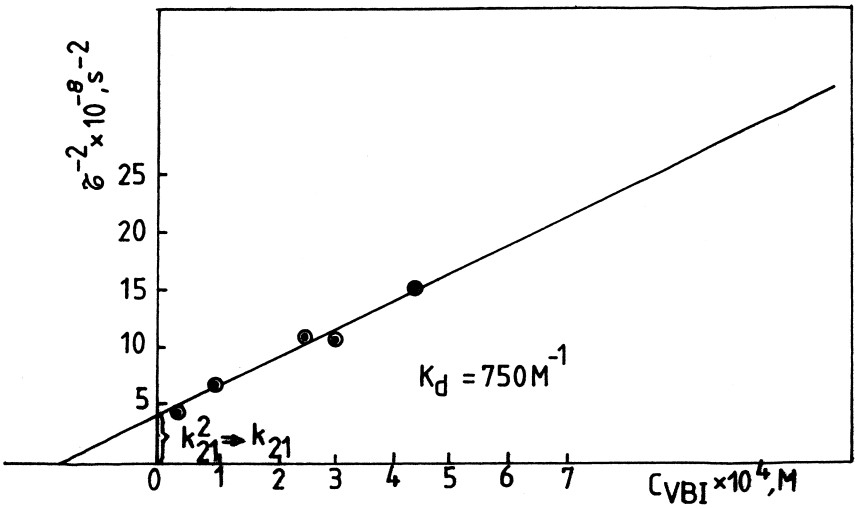

Fig. 1. The plot of the relaxation equation for dimerization.

As a reaction between two ions with positive charges, the dimerization was checked to see whether it is governed by the Bjerrum–Bröensted equation:

$$\log k = \log k^o + z^2 I^{1/2}/(1 + I^{1/2}) \quad (3)$$

in which k and k^0 are the rate constants at the ionic strength I and zero, respectively, and z the positive charge of violamycin molecule.

Fig. 2 represents the plot of this equation for the dimerization rate constant k_{12}. The positive slope confirms a reaction between species with the same sign of the charges.

The slope $z^2 \sim 5$ leads to $z = 2.24$, near to the charge $+2$ of violamycin BI. The intercept $k_{12}^o = 1.23 \times 10^7$ M^{-1} s^{-1} represents the extrapolated dimerization rate constant at the ionic strength $I \rightarrow 0$.

The dissociation rate constant k_{21} is found to be independent of the ionic strength, as shown in Fig. 3. The average value is $k_{21} = 4.13 \times 10^4$ s^{-1}. Their ratio gives a $K_d^o = 298$ M^{-1}

As a consequence, the equilibrium constant K_d follows the variation of the rate constant k_{12}, with the ionic strength. Fig. 4 shows this behavior and gives an extrapolated value $K_d = 300$ M^{-1} for $I \rightarrow 0$ like the ratio between k°_{12} and $k^\circ_{21,}$ 298 M^{-1}.

Separately we carried out spectrophtometric measurements in distilled water where VBI concentration assured an ionic strength of 8×10^{-4} M. The value obtained $K_d = 500$ M^{-1} is consistent with the above extrapolated one.

Table 2
The effect of ionic strength on the dimerization in phosphate–EDTA buffer at 20.5°C

a_{NaCl} M	I, M	$k_{12} \times 10^{-7}$ M^{-1} s^{-1}	$k_{21} \times 10^{-4}$ s^{-1}	K_d M^{-1}	
				T. jump	Spectroph.
0.000	0.020	1.50	2.00	750	732
0.025	0.045	9.05	5.87	1542	1499
0.050	0.070	17.41	5.53	3148	3400
0.100	0.120	21.15	3.36	6295	6150
0.200	0.220	–	–	–	9355

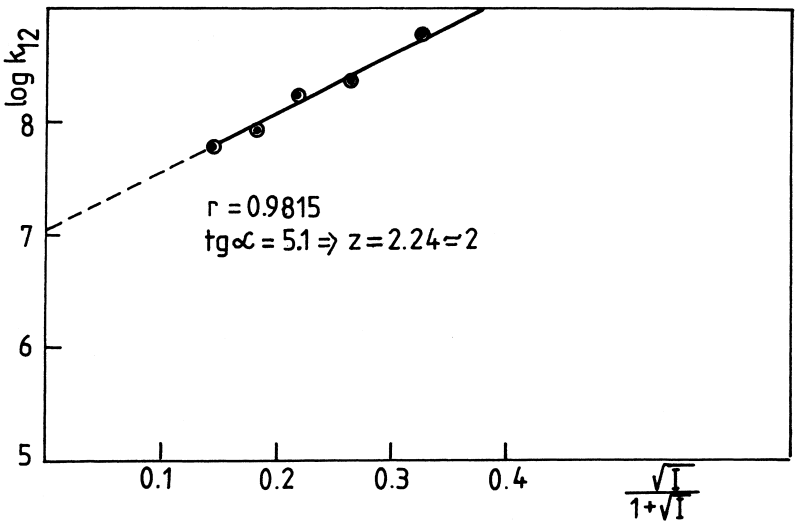

Fig. 2. The variation of the dimerization rate constant with the ionic strength.

In addition we evaluated also spectrophotometric the equilibrium constants at $I = 0.07$ M and $I = 0.12$ M in the same buffer to have a complete view on K_d values obtained by both used methods, listed in Table 2.

4. Discussion

In discussing these results it is important to note that ΔH_o for dimerization equilibrium is negative, although dimer formation is accompanied by a rather large electrostatic repulsion between the associating reaction partners, especially at the lowest ionic strengths. Other non-entropic binding forces leading to negative dimerization enthalpy must, therefore, be present. They include the energy lowering of the excited states of chromophores in the dimer (indicated by the red-shift of absorption band of the chromophores

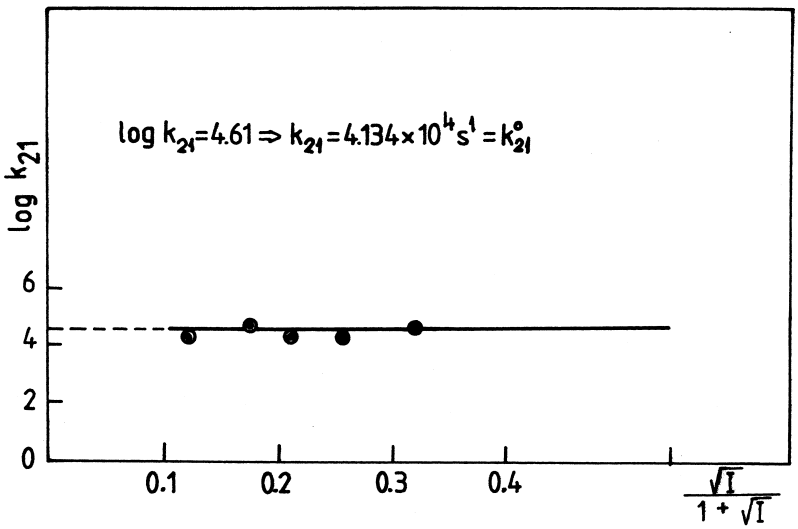

Fig. 3. The behavior of the dissociation rate constant of the dimer at various ionic strengths.

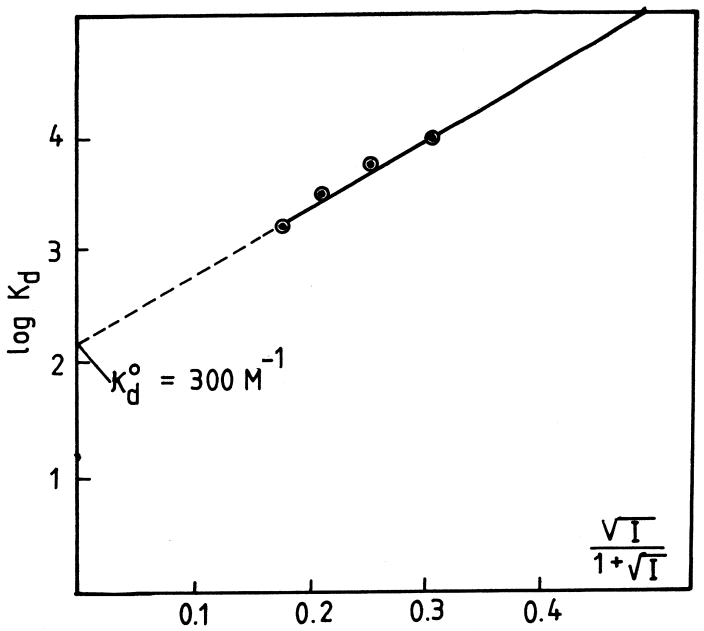

Fig. 4. The extrapolated value of the dimerization constant at $I \rightarrow 0$.

in the dimer), accompanied by a lowering of the transition dipole moments (the molar absorption coefficient of the monomer in the dimer is lower than that of the free dimer). In addition, local stable intermolecular H-bond formation with OH groups on the molecules may be involved, although these cannot be made responsible for large enthalpy changes since then the previously existing H-bonds with the solvent must be given up on binding.

The hydrophobic contributions from the release of water molecules from the contact surface of monomers in dimer are another driving force for dimerization, however, these contributions are mainly entropic at room temperature. They will add only a small amount to the negative enthalpy of dimerization because these forces are not very temperature sensitive at room temperature.

At very high ionic strength when the electrostatic repulsion is screened by the cloud of counter-ions, the bimolecular rate constant of association is still lower than that of diffusion controlled encounter rates of uncharged molecules of corresponding sizes, which may reach values near to 10^{-10} M^{-1} s^{-1}. The reaction mechanism of a bimolecular reaction in solution can be resolved into the formation of a short-lived encounter pair MM in which the monomers are in contact but may still have arbitrary orientations and a final step forming the stable dimer D as follows:

$$M + M \underset{k_{ba}}{\overset{k_{ab}}{\rightleftharpoons}} MM \underset{k_{cb}}{\overset{k_{bc}}{\rightleftharpoons}} D \tag{4}$$

with

$$k_{12} = k_{ab}\, k_{bc}/(k_{ba} + k_{bc})$$

and

$$k_{21} = k_{cb}\, k_{ba}/(k_{ba} + k_{bc})$$

The ionic strength, influencing the repulsive forces during diffusion approach, changes only the values of k_{ab} and k_{ba}. Since the measured k_{21} is independent of ionic strength, k_{bc} cannot be larger than a few percent of k_{ba}. One then has:

$$k_{12} = k_{ab}k_{bc}/k_{ba} \text{ and } k_{21} = k_{cb} \tag{5}$$

The equilibrium constant of encounter-pairs k_{ab}/k_{ba} follows the Bjerrum–Bröensted equation, leading to the validity of this relation for k_{12}. For uncharged particles, the encounter equilibrium constant is approximately 1 M^{-1}, if the size of the particles is such that their centers are separated by approximately 0.75 nm in the encounter complex [8]. For charged particles, with charges of equal sign, this value is smaller because k_{ab} decreases and k_{ba} increases due to the electrostatic repulsion. Finding $k_{bc} \ll k_{ba}$ indicates that an encounter pair, once formed, will with high probability, again diffuse apart before a stable dimer is formed. This means that some activation barrier, perhaps of entropic nature, must be overcome before the interacting monomers get in their stable dimeric configuration, in which they remain stabilized by the above mentioned enthalpic and entropic binding forces. We conclude that a specific mutual orientation of the monomers in the encounter, and perhaps a specific conformation of their side groups is required before a stabilizing action of the binding forces sets in.

5. Conclusions

Kinetics and thermodynamic of violamycin BI dimerization as well as the influence of the ionic strength on its characteristic parameters were never studied in the literature. Our results suggested also some theoretical considerations regarding the reaction mechanism of dimerization. It is supported by our experimental data interpreted in a manner, which was never applied to the dimerization mechanism.

Such investigations are necessary for our study regarding the kinetics of violamycin BI binding to poly-C under different ionic strength conditions.

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