The Self-Association of Bleomycin - a Chemical Basis for Superior Pharmacokinetics

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Bleomycin, a potent chemotherapeutic antibiotic, self-associates in aqueous solution at concentrations greater than 5 .10^{-6} M . We report in this paper visible spectrophotometric experiments that characterize this self-association. We started from a simple dimerization model supported by our experimental data and we used different methods described in literature for the computation of the molar absorption coefficient of monomer (ε_M), the molar absorption coefficient of dimer (ε_D) and the dimerization constant (K_d). These chemical properties of bleomycin result in superior pharmacokinetics, pharmacodynamics and, perhaps, therapeutic advantages.

Keywords: bleomycin, self-association, absorption spectroscopy

In modern society, stress could damage the quality of life. In this context, there are major concerns in order to improve drugs quality, by growing the amount of money spent for this reason. The main goal is to increase the drug efficiency and to reduce their possible secondary effects. Another important issue to be underlined is that new drugs penetrating the market must be characterized from the multiple perspectives of physico-chemical, structural and pharmacological properties. The drugs efficiency and physico-chemical properties depend essentially on their molecular and three-dimensional structure.

They were discovered numerous groups of new drug substances, which completely changed the therapy of the majority of diseases that formerly, represented outrageous flagellation. In spite of the remarkable progresses, it does not yet exist adequate causal medication for many important illnesses: senility, malignant tumors, AIDS etc. [1].

There long desired perfect drug "the magic pill", panacea, which has all the properties imposed by a modern medication, like controlled transformation, targeted transport to the organ or tissue that represents the place of action, a good tolerance, and absence of secondary effects, is far from being obtained, even for a single disease. To reach such objectives it is an intensive work, using the latest concepts in cell biology, pharmacology, biophysics and physical chemistry. The application of principles and knowledge accumulated in physical chemistry in the latter half of the century permitted the synthesis of new pharmaceutical formulations, increasing therapeutical performances and highlighting pharmaceuti-cal formulations as promising solutions for the contemporary pathology**.** Also, the physical chemistry principles supported the settlement of drug design on solid scientific bases, giving theoretical models for study that allow simulation of physiological active substances behavior interacting with living organisms.

There are complex interactions between drugs and organisms [1]. Following drug administration, there are three phases:

- pharmaceutical phase - that encloses disintegration of the pharmaceutics form and the dissolving of the active substance, processes that make the medicine available for absorption. It determines the so-called pharmaceutics availability, whose profile is defined by the time-concentration relation at the site of absorption.

- pharmacokinetic phase – that encloses absorption, distribution, metabolism and excretion of the medicine, determining its biological availability. The time-concentration relations in plasma and in target tissue (site of action) define the biological availability profile.

- pharmacodynamic phase – consists in drug-living matter interaction, in the target tissue, having as consequence the characteristic pharmacological effect.

The knowledge of pharmacokinetic profile, of drugs movements and changes in parameters in organism, of plasmatic concentration (eventually, tissue concentration) and effect relations, permits the rational selection of the most adequate route of administration, of dosing optimal regime having direct practical implications. The pharmaceutical availability depends, on one hand on the drugs physical-chemical properties and on the another hand on biochemical and physiological processes that appear after drugs administration.

The biological activity of drugs supposes physical-chemical interaction or chemical interaction with component molecules of living matter. The primary action, performed at molecular level acts on cell metabolism, influencing the structure, the biosynthesis and catabolism of nucleic acids, proteins, carbohydrates and lipids. A series of drugs inhibit the synthesis of nucleic acids precursors. Certain purine and pyrimidine derivatives incorporate in the nucleic acids, which, because of the presence of abnormal bases in their structure, result in biochemical dysfunction. A number of drugs important from clinical point of view interact directly with cell DNA and indirectly inhibit the replication and transcription. The precise mechanism that inhibits these functions is unique for every drug and may imply the RNA and DNA polymerase activity, topoisomerase II activity or other different enzymatic activities that use the DNA model. A fundamental step in understanding the molecular mechanism of drug action is the characterization of their binding to DNA equilibrium. Some drugs due their antibacterial activity to the interference in the protein synthesis by inhibiting or changing of translation genetic messages mechanisms.

Bleomycins, a family of natural occurring glycopeptides antibiotics, have a proven activity against testicular and

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prostatic cancers, skin carcinoma, Hodgkin's lymphoma, head and neck tumors [2]. The commercial form of these drugs, blenoxane, contains ca. 70% bleomycin A_2, 25% bleomycin B_2 and trace amounts of other congeners. Antitumoral activity of bleomycin is attributed to its ability to cleave single- and double- stranded DNA [3]; more recently, it was postulated that bleomycins could also cleave various types of RNA [4].

It is the aim of this paper to report absorption spectral experiments to study the dimerization equilibrium of bleomycin, in order to determine the molar absorption coefficient of monomer (ε_M), the molar absorption coefficient of dimer (ε_D) and the dimerization constant (K_d).

Experimental part

Bleocin (bleomycin hydrochloride) was obtained from Nippon Kayaku Co, Tokyo, Japan. Bleomycin solutions were prepared in aqueous medium. The concentrations of the solutions were determined using the molar absorption coefficient value: ε_{291nm}=15500 $M^{-1}cm^{-1}$. The absorption spectra were recorded in a Lambda 25 PerkinElmer spectrometer, with quartz cells, at room temperature.

Results and discussion

Bleomycin is one of the most investigated drugs used in cancer chemotherapy. Bleomycin was firstly isolated as a Cu^{2+}-containing glycopeptides antibiotic from the culture medium of *Streptomyces verticullus* and it was later found to be also an antiviral agent. It was soon found to be an anticancer agent. Similar to many other nature products, bleomycin is produced as a mixture of analogues with bleomycin A_2 and B_2 being the most abundant.

The therapeutic efficacy of drugs is believed to derive from its ability to bind to and oxidative degrade cellular DNA and double-strand DNA cleavage and the process is metal-ion and oxygen dependent [5, 6]. The structure of bleomycins (fig. 1) can be subdivided into three domains: the metal binding domain, the bithiazole moiety with a positively charged alkyl substituent attached to it and a disaccharide unit [7].

The first indication of bleomycin self-association is obtained from the spectrometric titration data, illustrated in Figure 2. Over the range 10^{-6}-$3\cdot10^{-4}$ M, the molar absorption coefficient seems to be constant at 15500 $M^{-1}cm^{-1}$, the molar absorption coefficient of monomeric bleomycin at 291nm. From the slope of the linear plot one obtains an ε_M value practically the same within our experimental errors 15500 ± 500 $M^{-1}cm^{-1}$.

Figure 3 presents the absorption spectra of the bleomycin solutions. One observes a band centered at 291nm and the shoulder at 310nm. It may be noted that the relative intensities of those bands vary upon dilution and that the absorption in the range 240-350nm is more pronounced at higher concentrations.

On this basis, and taking into account literature data on the aggregation of dyes or drugs [8-10], the band at 291nm

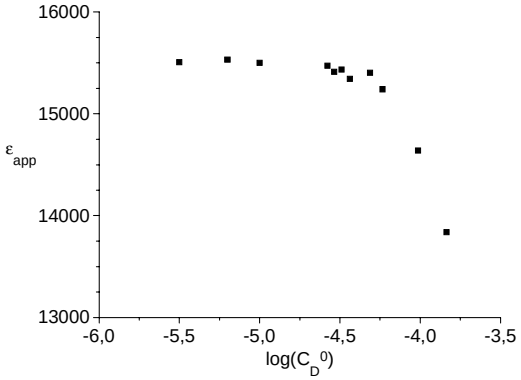


Fig. 2. Concentration dependence of molar absorption coefficient

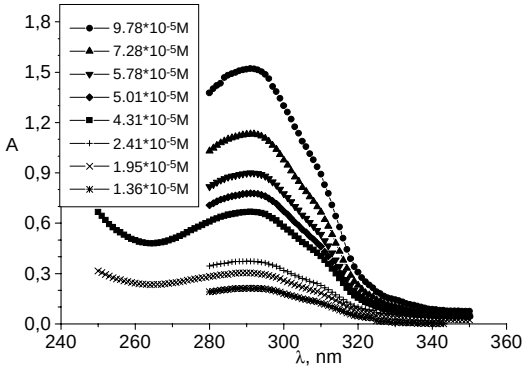
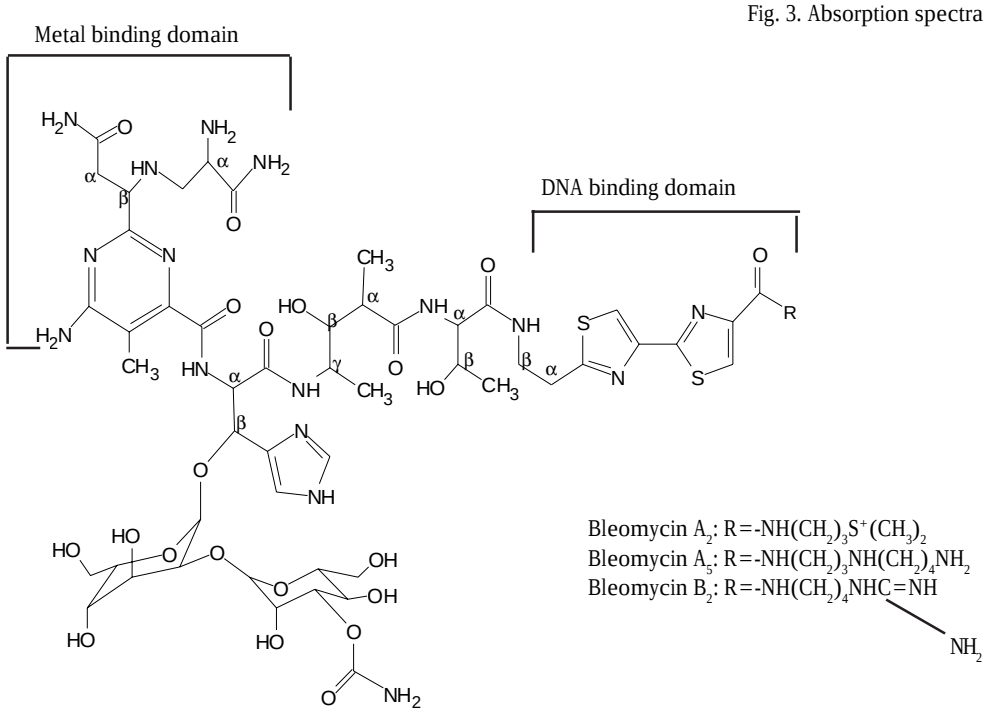


Fig. 3. Absorption spectra of bleomycin solutions



Bleomycin A_2: R = -NH$(CH_2)_3S^+(CH_3)_2$
Bleomycin A_5: R = -NH$(CH_2)_3$NH$(CH_2)_4NH_2$
Bleomycin B_2: R = -NH$(CH_2)_4$NHC(=NH)NH$_2$

Fig. 1. Structure of bleomycin-A_2, A_5 and B_2

was assigned to the monomer and the shoulder at 310 nm to the dimer.

The literature describes several computation methods [11,12] for estimating the dimerization constant. First, we calculated the dimerization constant K_d using the method described by Tipping et al [11] starting from the equilibrium monomer-dimer:

$$2M \rightleftharpoons D \quad (K_d)$$

and the following equations:

$$K_d = \frac{[D]}{[M]^2} \quad (1)$$

$$A = \varepsilon_{app} C_D^0 l = (\varepsilon_M [M] + 2\varepsilon_D [D]) l \quad (2)$$

where C_D^0, the total concentration of bleomycin in terms of monomer, is:

$$C_D^0 = \frac{[M]\varepsilon_M + 2[D]\varepsilon_D}{\varepsilon_{app}} \quad (3)$$

Rearrangement of the equation 2 gives:

$$C_D^0 = \frac{[M]\varepsilon_M + 2[D]\varepsilon_D}{\varepsilon_{app}} \quad (4)$$

At very low bleomycin concentrations, the monomer concentration greatly exceeds that of the dimer and in the limit $C_D^0 = [M]$ and $\varepsilon_M = \varepsilon_{app}$. Then extrapolation of a plot of $1/\varepsilon_{app}$ against concentrations of drug (fig. 4) to $C_D^o \rightarrow 0$ gives $1/\varepsilon_M$ as the intercept and gives a value of 15500 $M^{-1}cm^{-1}$ for ε_M.

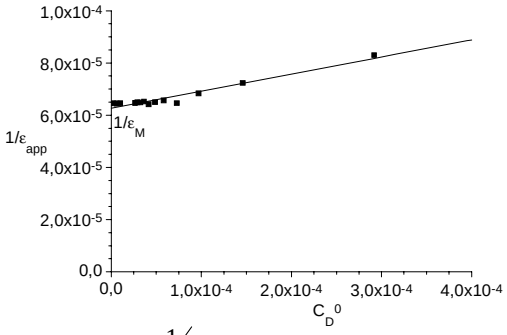


Fig. 4. The plot of $1/\varepsilon_{app}$ versus total concentrations of bleomycin

Elimination of [M] using (1) and (3), respectively (2) and (3), followed by elimination of [D] yields the expression:

$$\sqrt{\frac{C_D^0}{\varepsilon_M - \varepsilon_{app}}} = \frac{1}{\varepsilon_M - \varepsilon_D}\sqrt{C_D^0(\varepsilon_M - \varepsilon_{app})} + \sqrt{\frac{1}{2K_d(\varepsilon_M - \varepsilon_D)}} \quad (5)$$

Thus, a plot of $\sqrt{\frac{C_D^0}{\varepsilon_M - \varepsilon_{app}}}$ against $\sqrt{C_D^0(\varepsilon_M - \varepsilon_{app})}$ should give a straight line, the intercept and slope of which can be used to calculate K_d and ε_D.

The method proposed by Schwarz et al [12] may be used to obtain the dimerization constant K_d on the basis of the equation:

$$\sqrt{\frac{\varepsilon_M - \varepsilon_{app}}{c_D^o}} = \sqrt{\frac{2K_d}{\Delta\varepsilon}}[\Delta\varepsilon - (\varepsilon_M - \varepsilon_{app})] \quad (6)$$

where $\Delta\varepsilon$ is the difference in the molar absorption coefficients of the monomer and dimer. This equation is obtained by combining the equations (1), (2) and (3), e.g. by eliminating [M] and [D]. A linear plot of $\sqrt{\frac{\varepsilon_M - \varepsilon_{app}}{c_D^o}}$ versus $(\varepsilon_M - \varepsilon_{app})$ yields Δ_ε and K_d from x intercept and slope. Both methods lead to a dimerization constant of $K_d = 2400 \pm 100 M^{-1}$ and a molar absorption coefficient of the dimer $\varepsilon_D = 10800 \pm 500 M^{-1}cm^{-1}$.

Conclusions

The knowledge of basic spectroscopic properties of bleomycin can be exploited by investigating its interaction with biological macromolecules. This interaction competes with the equilibrium of self-association so, it is important to study the latter separately. One observes that, in the limit of the experimental errors K_d does not change and it pleads for the presence of a single species of aggregate, videlicet the dimer.

In conclusion, the research of drug searches fundamental problems of pharmacodynamics, pharmaco-toxicology and pharmacokinetics, having as primary objective the obtaining of some theoretical important data for the future development of the pharmacology, for the understanding of some general biological phenomena. The applicative part of the drug research explores at last the development of new medicines and evaluation in terms of efficacy and security of every therapeutic substances, having as direct purpose the wealth of the sick and the preservation of the health state. The considerable increase in the number of substances with perspectives of therapeutic utilization had raised the problem of performing careful studies, experimental and clinical. A new drug must go through several tough tests before being used as a therapy. For the present time, from approximately 100 compounds tested on animals, one arrives to clinical testing and from 45 compounds that had been clinically tested, only one has chances to become a drug. In addition, from 400 new formulations, alone or in combinations, which enter the pharmaceutical network, at global level, every year, few resist to the test of time.

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