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Complexing daunorubicin with β-cyclodextrin derivative increases drug intercalation into DNA

Agata Krzak[a], Olga Swiech[a], Maciej Majdecki[b], Renata Bilewicz[a,*]

[a] University of Warsaw, Faculty of Chemistry, Pasteura 1,02-093 Warsaw, Poland
[b] Institute of Organic Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

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ABSTRACT

A non-toxic lipoic acid derivative of β-cyclodextrin (βCDLip) with an electron-rich aromatic linker was studied as a carrier for the drug daunorubicin with the aim of increasing the intercalation efficiency of the drug into the double stranded DNA. The impact of cyclodextrins modified with lipoic acid on the interaction of daunorubicin with dsDNA was investigated by UV–vis spectroscopy and square wave voltammetry, and described using the McGhee and von Hippel model. The changes of the stability constants of the complexes reveal the dependence of drug binding efficiency on pH and explain the observed changes in the extent of drug intercalation into dsDNA.

1. Introduction

Physicochemical analysis of the factors affecting drug binding to nucleic acids is necessary in order to establish new ways of drug delivery to the target and to increase the efficiency of drug intercalation. The interaction between various small molecules and double stranded DNA (dsDNA) is, therefore, an important fundamental issue in life science related to the replication and transcription of DNA in vivo, the action mechanism of DNA-targeted drugs, or the action mechanism of synthetic chemical nucleases.

Daunorubicin (DNR) is an anthracycline antibiotic with antiblastic and anticancer activity. It is connected to the formation of intercalative complexes with dsDNA and the inhibition of both DNA and RNA synthesis [1]. The clinical effects are associated with modification of the DNA structure primarily through intercalating complexes into dsDNA and covalent bonding [2,3]. In particular, the tetracyclic A-D chromophore of these compounds is oriented with its long axis perpendicular to the long axis of adjacent base pairs at the intercalation site (Scheme 1).

The daunorubicin–DNA complex is stabilized by the stacking interactions of rings B and C, and by hydrogen bonding involving the hydroxyl group at C-9 of ring A, which acts as a donor to the N-3 of guanine and as an acceptor from the amino group of the same guanine. Alternatively, Ring D protrudes into the major groove and the amino sugar moiety lies in the minor groove and does not take part in the interaction with dsDNA, although it is crucial for antitumor activity. Similar to other antitumor intercalating agents, anthracyclines are topoisomerase II poisons because of the formation of a stable drug–DNA–topoisomerase II ternary complex and the consequent inhibition of both replication and transcription. The sugar unit is crucial for stabilization of this complex, and suppression of the C-4 methoxy and C-30 amino groups, thereby decreasing their activity, increases the topoisomerase II inhibition [4].

The specific toxicity of daunorubicin is due to the formation of reactive oxygen species (ROS) as a result of redox reactions involving anthracyclines [5,6]. Due to its very high reactivity, the ROS can interact with cellular components such as proteins, lipids, carbohydrates or nucleic acids. In particular, its impact on DNA is most dangerous since single- or double-stranded DNA undergo splitting, and nitrogen containing molecules can oxidatively modify the nucleic bases. All of these modifications can cause mutations [7,8]. The most disturbing and dangerous effect of anthracyclines is their cardiotoxicity. It has been shown that within one year after therapy with anthracyclines, dilated cardiomyopathy and chronic heart failure have generally developed [9], and within only a week of therapy initiation, one may develop acute cardiotoxicity [10]. It is believed that anthracyclines induce cardiotoxicity through other mechanisms than those responsible for anti-cancer activity. Research has shown that a decrease in the amount of active oxygen leads to a reduction in the cardiotoxic

* Corresponding author.
E-mail address: rbilewicz@gmail.com (R. Bilewicz).

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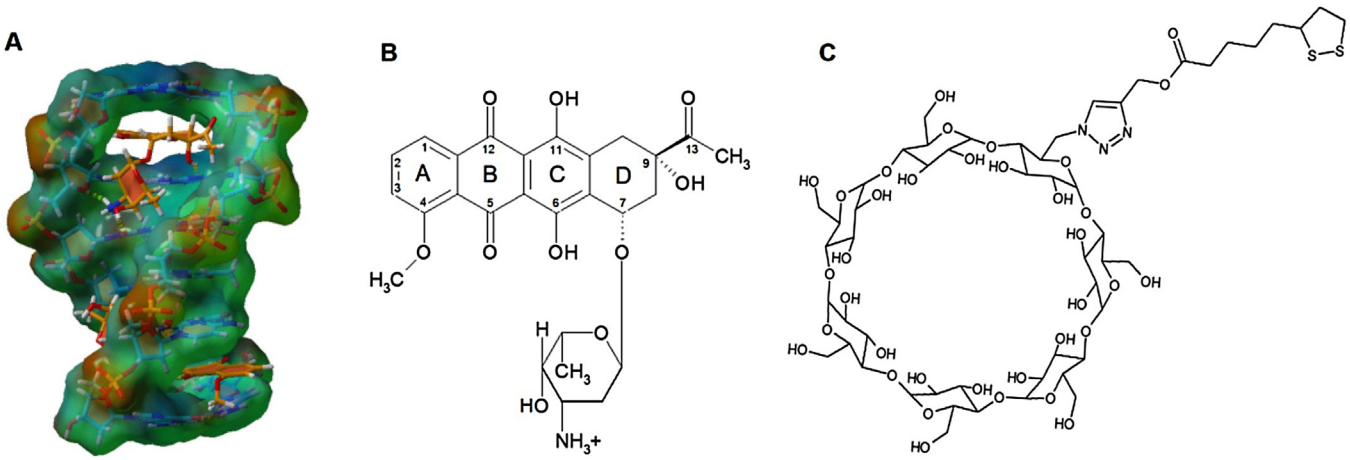

Scheme 1. The structure of the dsDNA – DNR intercalation complex (A), daunorubicin (B) and βCDLip (C).

effects of anthracycline [11]. On the other hand, the presence of the hydroxyl radical is necessary to create a covalent bond between the sugar group of anthracycline and the guanine group of the dsDNA, because without amine group the anti-cancer effect of the drug is limited.

To prevent the adverse action of ROS, the anthraquinone group of antracycline antibiotic can be blocked until the drug molecule is delivered to the pathologically changed cells. This protective effect can be achieved either by the complex formation between the anthracycline molecule and the cells, or by cyclic oligosaccharides such as cyclodextrins (CDs). However, such blocking must act selectively, in order to distinguish between the healthy and pathologically changed cells. Studies of tumor cells have shown that the pH of the interstitial fluid surrounding the tumor is lower than the pH of normal cells [12]. Following these studies, our group synthesized and examined the complex-forming abilities of new derivatives of β-cyclodextrins possessing either an aromatic group or antioxidant (lipoic acid) connected via an aromatic triazole group. Voltammetric and spectroscopic studies revealed that these two types of carriers can be employed for the pH-selective binding of anthracycline drugs. At pH 7.4, the new derivatives form strong complexes with doxorubicin, whereas at pH 5.5 the stability constants are much lower [13,14].

Apart from their blocking abilities, cyclodextrins can exhibit properties of antitumor action and support drug binding to dsDNA. Finance et al. studied the ability of antracycline – cyclodextrin complexes to enhance the antitumor activity of selected drugs [15]. They observed a 10-fold increase in the antitumor activity of doxorubicin in the presence of native βCD. Uekama et al. discovered that the presence of γCD delayed drug release from the liposomal carriers and limited the retraction of the drug from cancer cells, thereby increasing retention [16]. Ding et al. investigated the interactions of daunorubicin with herring sperm dsDNA in the presence of native γcyclodextrin. The authors showed that the intercalation of γ-CD–DNR into dsDNA increased with the increasing concentration of the cyclodextrin [17]. The supporting effect of the cyclodextrin leading to the increase of the drug-DNA intercalation is due to the specific interactions of cyclodextrin with double stranded DNA. Rocha et al. have shown that native βCD can locally denature the DNA molecule by forming hydrogen bonds with the base pairs resulting in relaxation of the DNA structure [18]. This kind of interaction can be enhanced by modification of the cyclodextrin with a functional group, such as a cationic amine moiety [19].

Our goal was to investigate the influence of cyclodextrins modified with lipoic acid (Scheme 1C) on the interaction between daunorubicin and dsDNA. Cyclic voltammetry and square wave voltammetry were used to estimate the stability constants of the cyclodextrin-daunorubicin inclusion complex. UV–vis spectroscopy and square wave voltammetry allowed for tracking the changes of daunorubicin intercalation into dsDNA in both the absence and presence of the cyclodextrin ligand.

2. Experimental

2.1. Chemicals and reagents

Daunorubicin (DNR) hydrochloride salt was purchased from AK Scientific. Calf thymus (ct) double stranded DNA (dsDNA) was purchased from Sigma. The dsDNA solutions of 1 mg dsDNA per 1 mL of Britton-Robinson (BR) buffer were prepared at least 24 h before the experiments. The purity of dsDNA was assessed by monitoring the absorbance ratios at different wavelengths $A_{\lambda=260nm}/A_{\lambda=280nm}$ and $A_{\lambda=260nm}/A_{\lambda=250nm}$, which should be in the range 1.7–2.0 and 1.4–1.7, respectively [20]. The dsDNA solutions had absorbance ratios in the middle of these ranges. The synthesis of the lipoic acid derivative of β-cyclodextrin (βCDLip) was performed as previously described [14]. Other compounds were purchased from Aldrich or Fluka. Buffers were prepared using water from a Milli-Q ultrapure water system. Britton-Robinson buffers (pH 7.4 and 5.5) were prepared in the usual way by the addition of appropriate amounts of 0.2 M sodium hydroxide to orthophosphoric acid, acetic acid and boric acid (0.04 M solutions). The pH was controlled using a pH-Meter E2 (Mettler Toledo).

2.2. UV–vis spectroscopy

The absorption spectra were recorded using the Cary 60 spectrophotometer (Agilent Technologies) with 1 cm path-length quartz cuvettes. The concentration of dsDNA was determined from the value of the absorbance measured at λ = 260 nm, ε = 13200 $M^{-1}\,cm^{-1}$ [21]. The absorbance of dsDNA vs. concentration plots were perfectly linear in the range 20–70 μM (y = 0.1320 × −0.1658 × 10^{-6}, r = 0.9999), demonstrating the homogeneity of the dsDNA solutions.

2.3. Voltammetry

Square wave voltammetry (SWV) experiments were performed using an EC Epsilon potentiostat (BASI). The electrochemical cell was kept in a Faraday cage. All electrochemical experiments were