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Influence of Cr(III) and Cr(VI) on the interaction between sparfloxacin and calf thymus DNA

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Abstract

Cr(III) and Cr(VI) have different binding capacity with sparfloxacin, and have different combination modes with calf thymus DNA. Selecting these two different metal ions, the influence of them on the binding constants between sparfloxacin (SPFX) and calf thymus DNA, as well as the related mechanism has been studied by using absorption and fluorescence spectroscopy. The result shows that Cr(III) has weaker binding capacity to SPFX in the SPFX–Cr(III) binary system, but influences the binding between SPFX and DNA obviously in SPFX–DNA–Cr(III) ternary system. However, although Cr(VI) has a stronger binding capacity to SPFX, it has no effect on the binding between SPFX and DNA. Referring to the different modes of Cr(III) and Cr(VI) binding to DNA, the mechanism of the influence of metal ions on the binding between SPFX and DNA has been proposed. SPFX can directly bind to DNA by chelating DNA base sites. If a metal ion at certain concentration binds mainly to DNA bases, it can decrease the binding constants between SPFX and DNA through competing with SPFX. While if a metal ion at certain concentration mainly binds to phosphate groups of DNA, it can increase the binding constants by building a bridge between SPFX and DNA. If a metal ion at certain concentrations binds neither to bases nor phosphate groups in DNA, it will have no effect on the binding constant between SPFX and DNA. Our result supports Palumbo's conclusion that the binding between SPFX and the phosphata groups is the precondition for the combination between SPFX and DNA, which is stabilized through stacking interactions between the condensed rings of SPFX and DNA bases.

Keywords: Sparfloxacin; Calf thymus DNA; Cr(III); Cr(VI)

1. Introduction

Quinolones are a group of synthetic antibacterial agents containing a 4-oxo-1, 4-dihydroquinoline skeleton. In general, quinolones can act as antibacterial drugs that effectively inhibit DNA replication and are commonly used as treatment for many infections [1,2]. Compared with first- (nalidixicacid, cinoxacin) and second- (norfloxacin, enoxacin, ofloxacin, and ciprofloxacin) generation, third-generation quinolones (levofloxacin, sparfloxacin, gatifloxacin, and moxifloxacin) show a much broader spectrum of activity providing expanded gram-negative and gram-positive activity coverage as well as expanded activity against atypical pathogens [2–4]. The interaction of metal ions (M) with diverse first- and second-generation quinolones as ligands, and the influence of the metal ions on the binding between the quinolones and DNA have been studied [5–12]. Sparfloxacin (SPFX) is a third-generation quinolone antimicrobial drug, which is mainly used for the treatment of acute exacerbations of chronic bronchitis and community-acquired pneumonia [2]. Although electrometric studies, elemental analysis and magnetic measurements of a parrot-green inner complex SPFX–copper(II) have been reported [2], the interaction between metal ions and SPFX and the influence of metal ions on the binding between SPFX and DNA have not been reported yet.

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Quinolone develops its pharmacological action via specific inhibition of sub-unit A of the bacterial gyrase, which is an enzyme controlling DNA shape [13]. Although the exact mechanism of this action is still unclear, there is evidence that quinolone interacts directly with DNA in synergy with the gyrase enzyme [14,15]. Such interaction undoubtedly contributes to the desired antibacterial activity. However, it can also be responsible, at least in part, for the unwanted toxic effects [11]. Therefore, contributions to deeper insight into the interaction mechanism of this antibiotic class with DNA might be important for a better understanding of their therapeutic efficacy.

Höffken et al. first reported that concurrent administration of magnesium–aluminum containing antacid with ciprofloxacin almost resulted in a complete loss of activity of the drug in serum. Some authors studied the reasons of the reduced activity of quinolones with the presence of the ions [5,16,17]. According to these results, it was suggested that the multivalent cations should be avoided in patients receiving quinolone antibacterials. At the same time, the proposed mechanism of interaction between quinolone and metal cations was chelation between the metal and the 4-oxo and adjacent carboxyl groups. Since these functional groups are required for antibacterial activity, it could be anticipated that all of the quinolones will interact with metal ions. However, as some authors observed, there might be differences between the quinolones and metal ions regarding the extent of interaction [18].

Palumbo et al. stressed the role of magnesium in the quinolone–DNA interaction [19,20]. It was suggested that Mg(II) acts as a bridge between the quinolone and the phosphate group of DNA, and that this complex is stabilized by stacking interactions between the condensed rings of the drug and the DNA bases in a single-stranded region or distorted B-form in plasmid. From a theoretical-experiment study on the structure and activity of certain quinolones and the interaction of their Cu(II) complexes on a DNA model, it was suggested that the intercalation of the quinolone complexed to a metal is an important step in these processes [21].

So although the influence of metal ions on the binding between quinolones and DNA has been researched for many years, the action of metal ions in the interaction between quinolones and DNA is still unclear. One important subject is that whether the relative binding capacity of metal ion to phosphates as well as bases in DNA has any relationship with its action of effect on the binding between quinolone and DNA. In this article, we selected Cr(III) and Cr(VI), which have different ratios of affinity for phosphate to heterocyclic base in DNA [22,23], to study the influence of them on the binding between SPFX and calf thymus DNA. This study may provide useful information about the relationship between the binding mode of metal ions to DNA and the influence ability of metal ions on the binding between quinolones and DNA.

2. Materials and methods

2.1. Materials

SPFX (LKT labs, Inc, Japan) was kept solid in dark at 4 °C. A 0.02 M NaCl stock solution of SPFX was prepared immediately prior to use, and the concentration of SPFX in the solution was 1.0×10^{-3} M. Cr(III) and Cr(VI) from $CrCl_3$ and Na_2CrO_4 used in the experiment were obtained from Tianjin Chemical Reagents Company of China, and the concentrations of Cr(III) and Cr(VI) in the 0.02 M NaCl stock solutions were 0.1 M [10]. Calf thymus DNA (Sigma) was prepared by dissolving the DNA in 0.02 M NaCl solutions. DNA concentrations were expressed as DNA phosphate (DNA P) with a molar extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm [8,12].

2.2. Methods

The absorption and fluorescence spectra were obtained by using UV-265 spectrophotometer and RF-540 fluorescence spectrophotometer (Shimdazu, Japan). In fluorescence mode, both excitation and emission bandwidths were set at 10 nm. All of the spectroscopic work was carried out at ambient temperature and at pH 7.0 remained by a Tris–HCl buffer (0.01 M) [8,12].

The SPFX–DNA, SPFX–Cr(III) and SPFX–Cr(VI) binary systems were formed by titrating SPFX solution with DNA, $CrCl_3$ and Na_2CrO_4 solutions, separately. The concentration of SPFX was maintained at 2.0×10^{-5} M in absorption spectra determination and at 1.0×10^{-5} M in fluorescence spectra determination. The SPFX–DNA–Cr(III) and SPFX–DNA–Cr(VI) ternary systems were formed by titrating the SPFX–DNA binary system with Cr(III) and Cr(VI) solutions. The molar ratios of SPFX to DNA were varied from 0.07 to 0.4. During the titration operations, DNA, $CrCl_3$ and Na_2CrO_4 stock solutions were added in microliter quantities.

3. Results and discussion

3.1. Binary binding of SPFX with DNA, Cr(III) and Cr(VI)

In the absorption spectra, SPFX solution has an absorbance peak at 370 nm. Titrating SPFX solution with DNA, Cr(III) and Cr(VI) in increasing amount separately, causes a decrease in the absorbance, and a sharpening and shift to lower energy in the absorption band associated with the chromophore of the drug (Figs. 1–3). The titration is characterized by forming isosbestic points at 395 nm with DNA, and at 291 nm with Cr(III). These results indicate that SPFX can bind with DNA, Cr(III) and Cr(VI), and the bound chromophore is converted to a new species.

Under the aggregation concentration, 1.0×10^{-5} M SPFX was fixed and DNA was varied from 5.03×10^{-5} to 3.02×10^{-4} M by titrating SPFX with DNA. The