A Thermodynamic Study on the Interaction of Quinolone Antibiotics and DNA

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Fluorescence of quinolones including norfloxacin, ciprofloxacin and *S*- and *R*-ofloxacin is quenched upon association with single and double-stranded DNA (ss- and ds-DNA). The ratios of fluorescence intensity in the presence of DNA to its absent were plotted with respect to the DNA concentration to construct the Stern-Volmer plot. The slope of the Stern-Volmer plot become larger as the temperature is lowered, ensuring that the fluorescence quenching is static process, *i.e.*, the fluorescence is quenched by formation of the non-fluorescent complex between quinolone and DNA. In the static quenching mechanism, the quenching constant which is equivalent to the slope of the Stern-Volmer plot, is considered as the equilibrium constant for the association of quinolones and DNA. From the temperature-dependent equilibrium constant, ΔH^0 and ΔS^0 was obtained using the van't Hoff relation. In general, association of the quinolone with ds- as well as ss-DNA is energetically favorable (an exothermic) process while the entropy change was unfavorable. Due to the steric effect of the substituents, the effect of the quinolone ring is smaller on the ss-DNA compared to ds-DNA.

Key Words: DNA, Quinolone, Entropy, Enthalpy, Fluorescence

Introduction

Quinolone antibiotics have been increasingly being used for the treatment of various infectious diseases.[1] The acting target of these antibiotics has been known to be DNA gyrase, a bacterial type II DNA topoisomerase which convert the relaxed super-coiled DNA into a negatively super-coiled form. However, norfloxacin, a member of quinolone family did not bind directly to gyrase but it formed a complex with DNA.[2-5] although ciproxacin was reported to affect the conformation of DNA gyrase A in the presence of Mg^{2+}.[6] In the complex, norfloxacin proposed to bind in cooperative manner in the single stranded DNA pocket induced by gyrase. Importance of understanding the DNA binding properties of quinolones is highlighted by the finding that the binding of *S*-ofloxacin to DNA is far more preferable than *R*-enantiomer.[7,8] Considering that *S*-ofloxacinis is 8-128 times more potent than *R*-ofloxacin[9], this finding implies the relationship between the binding mode and preferentiality of quinolone antibiotic toward DNA and the antibiotic activity of quinolones.

In was reported that norfloxacin bind to DNA phosphate group in the presence of Mg^{2+} ion.[9] In the adduct, the carbonyl and carboxylic group of norfloxacin and two phosphate groups of DNA are chelated to a Mg^{2+} ion: thus, in the complex, Mg^{2+} ion takes a role as a bridge between norfloxacin and DNA. Binding mode of quinolone to DNA in the absence of Mg^{2+} ion which was investigated by spectroscopic as well as theoretical methods also has been reported[10-16] and can be summarized as (1) quinolone binds at the minor groove of double stranded DNA (hereafter referred to as ds-DNA) with possibility of partial intercalation, (2) in the binding site, the carbonyl and carboxylic acid moieties of quinolone form hydrogen bonds, especially, with guanine base which consequently explains the preferentiality of quinolone toward GC base pairs than AT base pairs, (3) the binding is more favorable to single stranded (denatured) DNA (hereafter referred to as ss-DNA). The origin of the binding mode has been partially elucidated by theoretical study. For instance larger binding affinity of *S*-ofloxacin than *R*-isomer was explained by the steric effect in the narrow minor groove. However, the quinolone-DNA interaction has not been fully understood. In this work, the thermodynamic aspect of formation of the complex between DNA and various quinolones namely, ciprofloxacin, norfloxacin, *S*-ofloxacin and *R*-ofloxacin (Figure 1) is investigated by fluorescence technique.

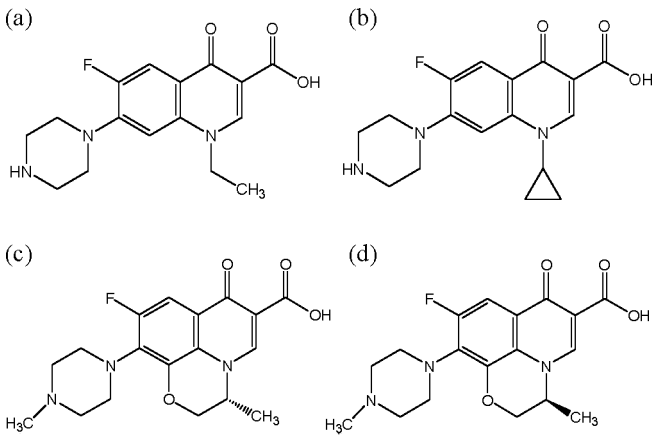

Figure 1. Chemical structures of quinolone antibiotics. (a) Norfloxacin (b) Ciprofloxacin (c) *R*-ofloxacin (d) *S*-ofloxacin.

Experimental

Material. Norfloxacin and racemic-ofloxacin were purchased from Sigma-Aldrich and ciprofloxacin from Buchs (Switzerland). *S*- and *R*-ofloxacin were separated using a HPLC (Shimadzu, LC10A, Japan) equipped with Chiralcel OD-H. The flow rate of eluent consists of hexane: ethanol: acetic acid = 60:40:0.5 was 0.7 mL/min. DNA purchased from Sigma-Aldrich was dissolved in a 5 mM cacodylate buffer, pH = 7.0, containing 1.0 mM EDTA and 100 mM NaCl by exhaustive shaking at 4 °C followed by several rounds of dialysis against 5 mM cacodylate buffer pH, 7.0. The latter buffer was used throughout this study. The concentrations of the quinolones and DNA were determined spectrophotometrically using the molar extinction coefficient: ε_{273nm} = 37500 $cm^{-1}M^{-1}$, ε_{288nm} = 30500 $cm^{-1}M^{-1}$, ε_{275nm} = 35900 $cm^{-1}M^{-1}$ and ε_{258nm} = 6700 $cm^{-1}M^{-1}$ for norfloxacin, ofloxacin, cirpfloxacin and DNA, respectively. Ss-DNA was obtained by simmering the ds-DNA for an hour followed by rapid cooling in ice-water. Ss-DNA was prepared prior to each measurement to prevent the self association.

Equilibrium measurement by fluorescence technique. Any process in which fluorescence intensity of a given fluorophore decreases by addition of a chemical species is called fluorescence quenching.[17] When the fluorescence intensity of a given flurophore is quenched by a quencher molecule by a simple static or dynamic mechanism, the ratio of the fluorescence intensity in the absence of quencher (F_0) to its presence (F) with respect to the concentration of the quencher ([Q]), which is called the Stern-Volmer plot, will appear to be a straight line.[36]

$$\frac{F_0}{F} = 1 + K[Q] \tag{1}$$

Where K is the Stern-Volmer quenching constant. In the static process, the fluorescence intensity of given fluorophore is quenched by forming a non-fluorescent complex with quencher molecule while the excited energy transfers by collision in the dynamic process. These two processes can be easily distinguished. In the static case, lowering the temperature enhances the process because the fluorophore-quencher complex formation is usually endothermic. The fluorescence decay time in the absence and presence of quencher is expected to be the same because the only species that fluorescent is quencher unbound fluorophore. In the dynamic quenching case, however, temperature dependence would be opposite. The rate of collision thereby, the probability of the energy transfer would increase as the temperature increase. The fluorescence decay time shorten because the energy transfer occurs during the fluorophore is excited. Excited quinolones give out its energy by fluorescing in the range of 400 ~ 600 nm and its fluorescence intensity deceases in the presence of native and synthetic DNAs.[7,8,11-16,18] That the fluorescence intensity of quinolone is quenched by various DNA has been known to occur through the static mechanism. In this case, the static quenching constant is equivalent with the equilibrium constant for the quinolone-DNA complex formation.

$$\text{Quinolone} + \text{DNA} \rightleftharpoons \text{DNA-quinolone complex} \tag{2}$$

The fluorescence intensity of the quinolones in the presence and absence of DNA was at 325 nm, and the emission was monitored at 415 nm. The slit widths were 8 nm and 12 nm, respectively, for excitation and emission. For the steady state fluorescence intensity measurement, a Jasco FP777 spectroflurometer was used. The temperature was kept constant by circulating water. The temperature in the cell was monitiored by a thermo-couple.

Analysis of data and obtaining thermodynamic parameters. Given temperature dependent equilibrium constants for the DNA-quinolone complex formation, it is easy to obtain the entropy, enthalpy and Gibb's free energy at a given temperature utilizing the temperature dependence of K_{eq} from the fundamental thermodynamic relationships, and from the van't Hoff equation.

$$\ln K_{eq} = -\left(\frac{\Delta H^\circ}{R}\right)\left(\frac{1}{T}\right) + \frac{\Delta S^\circ}{R} \tag{3}$$

$$\Delta G^\circ_{298} = -RT \ln K_{eq} \tag{4}$$

When the measured $\ln K_{eq}$ is plotted with respect to $1/T$, where T is temperature in K, the thermodynamic parameters namely, the standard enthalpy change, ΔH^0, and the standard entropy change, ΔS^0, can be calculated from slope and y-intercept. In the equation, R denotes molar gas constant.

Result and Discussion

Fluorescence quenching. The fluorescence emission spectrum of one of the quinolone namely, ciprofloxacin in the presence and absence of DNA is depicted in Figure 2. In the absence of DNA, ciprofloxacin exhibited a maximum at 416 nm in the fluorescence emission spectrum. That observed for other quinolones are similar to ciprofloxacin therefore are not shown. Upon addition of ds- and ss-DNA, the intensity of ciprofloxacin decreased. The extent of decreasing in fluorescence intensity is larger in the presence of ss-DNA and the appearance of fluorescence emission spectrum in the presence of either ss-DNA or dsDNA is identical to that in the absence of DNA. Considering that the fluorescence quenching is static in its nature (see below), the association of quinolones with ss-DNA is more effective than ds-DNA. This observation is in contrast with that reported by others,[13] and we do not have any explanation for this discrepancy.

As the concentration of either ds- or ss-DNA increased, the fluorescence intensity gradually decreased for all quinolones. The decrease in fluorescence intensity can be conveniently showed by the Stern-Volmer plot in which the ratio of the fluorescence intensity in the absence to that in the presence is depicted with respect to the quencher concentration. The Stern-Volmer plot for ciprofloxacin with ds-DNA as a quencher at various temperatures is depicted in Figure 3, as an example. At all temperatures, the Stern-Volmer plot appeared to be a

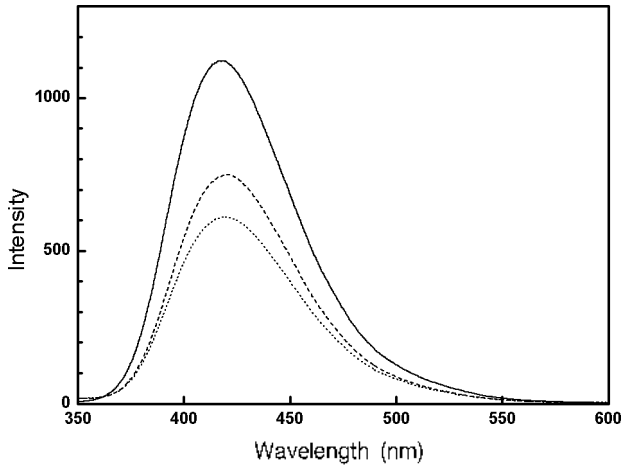

Figure 2. Fluorescence emission spectrum of ciprofloxacin in the absence (solid curve) and presence of ds- (dotted curve) and ss-DNA (dashed curve). [Ciprofloxacin] = 1 μM, [DNA] = 100 μM in base. Sample was excited at 325 nm. Slit widths are 10/10 nm for both excitation and emission

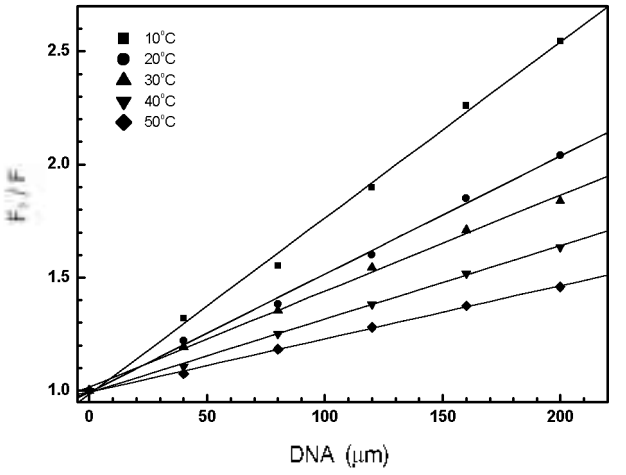

Figure 3. The Temperature-dependent Stern-Volmer quenching plot for the ds-DNA-ciprofloxacin complex formation. The concentration and the condition for the fluorescence intensity are the same as in Figure 2, except for DNA concentration.

straight line and the slope became smaller as the temperature increased. Appearance of the Stern-Volmer plot for other quinolones namely norfloxacin, *S*- and *R*-ofloxacin was similar, except for the slope of the straight line. A straight line in the Stern-Volmer plot indicates that the fluorescence quenching of ciprofloxacin by ds- or ss-DNA occurs through simple static or dynamic mechanism. In the former mechanism, fluorescence of a given fluorophore is quenched by formation of a non-fluorescent complex with quencher, in the current case, ciprofloxacin and DNA, respectively. On the other hand, dynamic quenching occurs by collision between excited flurophore and the quencher: the excited energy of the fluorophore transfer to the quencher in the collision process. These two mechanisms are easily distinguished by either temperature-dependent quenching efficiency or fluorescence decay time measurement or both. In the static quenching case, the formation of the non-fluorescent complex between fluorophore

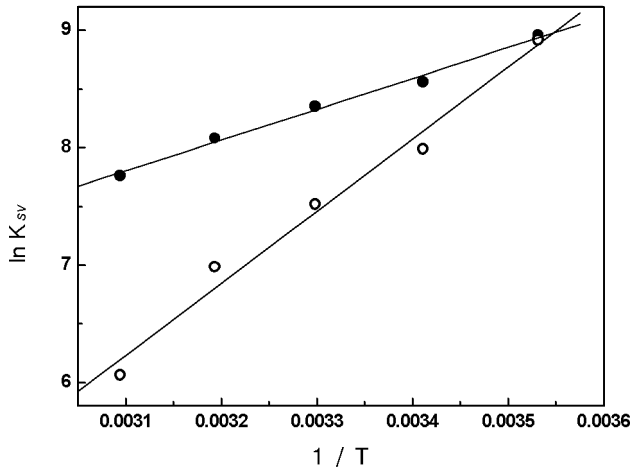

Figure 4. The van't Hoff plot for association of ciprofloxacin with ss- (open circles) and ds-DNA (closed circle) according to equation (4).

and quencher is often thermodynamically exothermic. Therefore, the formation of the complex is less effective at a high temperature, resulting in a smaller slope in the Stern-Volmer plot. The characteristics of the fluorescence of the non-complexed fluorophore remains therefore, the fluorescence decay times in the presence and absence of the quencher are identical. On the other hand, increase in temperature results in higher probability of collision between excited fluorophore and the quencher, which, in turn, results in more effective quenching at higher temperature in the dynamic mechanism. The fluorescence decay time is expected to be shortened because the excited energy of the fluorophore transfers to quencher while it is excited.

Thermodynamic parameters. The facts that the fluorescence decay times of norfloxacin have been reported to be the same in the presence of various DNAs[13] and that the quenching efficiency decreases as the temperature increases led us to conclude that mechanism behind the quenching of the quinolones' fluorescence by DNA is static. In the static process, the quenching constant which corresponds to the slope of the Stern-Volmer plot may be considered as the equilibrium constant for the formation of the fluorophorequencher complex (equation 2). For instance, the equilibrium constants obtained from association of ciprofloxacin to ds-DNA was 7790 M^{-1} and 2350 M^{-1} at 10 °C and 50 °C, respectively. Given the equilibrium constant at various temperatures, various thermodynamic parameters, *i.e.*, ΔH, ΔS and ΔG for the association of quinolone with DNA can be obtained according to the basic thermodynamic relation and the van't Hoff plot which is the log of the equilibrium constant relative to the reciprocal temperatures. In the calculation, the enthalpy and entropy change for the association of quinolone with DNA is assumed to be temperature-independent and this assumption may be valid for the narrow temperature range adopted in this study being 10 ~ 50 °C. The van't Hoff plot is depicted in Figure 4 for the equilibrium of the ciprofloxacin and ds- and ss-DNA as an example. In this particular instance, for the association of ciprofloxacin with ds-DNA, ΔH^0 is -21.9 kJmol^{-1} and ΔS^0 is -2.9 Jmol^{-1}K^{-1}. ΔH^0 with ss-DNA is -51.1 kJmol^{-1} and ΔS^0 is -106.7 Jmol^{-1}K^{-1}. In both cases, the association is driven by

Table 1. Summary of the thermodynamic parameters for the quinolones-DNA complex formation

Drug	DNA Strand	K_{sv} (M^{-1})	ΔH^0 ($kJ \cdot mol^{-1}$)	ΔS^0 ($J \cdot mol^{-1} \cdot K^{-1}$)	ΔG^0 ($kJ \cdot mol^{-1}$)
Norfloxacin	single	3970	-50.7	-103.4	-20.2
	double	4490	-26.0	-18.4	-20.5
Ciprofloxacin	single	2950	-51.1	-106.7	-19.5
	double	5220	-21.9	-2.9	-20.9
S-ofloxacin	single	2580	-78.2	-202.2	-19.1
	double	1710	-38.5	-68.8	-18.1
R-ofloxacin	single	1110	-57.4	-137.1	-17.1
	double	746	-30.9	-50.1	-16.1

the favorable enthalpy change while the entropy changes are unfavorable. The change in Gibb's free energy at 20 °C is -19.5 $kJmol^{-1}$ and -20.9 $kJmol^{-1}$ for ss- and ds-DNA, respectively showing that the association is thermodynamically favorable.

The thermodynamic parameters for association of all four quinolones with ds- and ss-DNA are summarized in Table 1. For all cases, change in the Gibb's free energy is negative indicating that the complex formation is spontaneous. Association is driven by favorable ΔHs (the negative enthalpies) and, therefore, is exothermic, while the entropy changes are unfavorable (negative entropies). At a glance of the table, the enthalpy change is larger for ss-DNA compared to ds-DNA, suggesting that the complex formed between quinolones and ss-DNA is energetically more stable than that formed with ds-DNA while the entropy change of the complex formation is far more unfavorable in the ss-DNA case. The unfavorable entropy with ss-DNA may be attributed to the conformation change of the ss-DNA upon quinolone binding: ss-DNA is more flexible than ds-DNA, therefore, the conformation of DNA near the quinolone binding site has larger possibility to change compared to ds-DNA. From the structural studies,[15,16,19] it was reported that the carbonyl and carboxylic groups of the quinolone forms hydrogen bonds with the G or A bases of DNA when form a complex with ds-DNA, which contributes the negative enthalpy. As a consequence, the carbonyl and carboxylic groups direct toward the center of DNA, and the part at which various derivatives were introduced partially exposed to solvent. In this regard, that effect of the substituents at the N atom of the quinolone ring, *i.e.*, ethyl, cycropropyl and oxazine ring for norfloxacin, ciprofloxacin and ofloxacin, respectively, on the thermodynamic parameters is less pronounced in the ss-DNA case with the exception of *S*-ofloxacin is understandable. The extent of the contact of the substitieunts with the DNA stem is less in the ss-DNA compared to ds-DNA, resulting in a similar thermodynamic parameters in the ss-DNA case. In contrast, a large deviation in the thermodynamic parameters, particularly the entropy change was noticed in the ds-DNA case. The large deviation conceivably is the result of different steric hindrance provided by the substituents, which is in contact with the wall of the DNA minor groove.[19]

Conclusion

Fluorescence intensity of various quinolone decreases upon binding to ss- and ds-DNA. Extent of decrease is larger at the lower temperature suggesting that the fluorescence quenching is static in its nature. All tested quinolone produced the positive slope and intercept in the van't Hoff plot. These observations suggests that association of the quinolone with ds- as well as ss-DNA is energetically favorable (an exothermic) process while entropy change is unfavorable. Effect of the substituents at the quinolone ring is smaller in the ss-DNA case compared to ds-DNA due to the steric effect of the substituents.

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