Investigation on the selective interactions between flouroquinolonyl -ampicillin derivative and double stranded oligonulceotides

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Abstract

The synthesized compound N-(6-fluoroquinoloyl)-ampicillin (FQ-1) exhibits a broad-spectrum on biological effects against bacteria, protozoa, virus and cancer cells. From the results of the migration shift of eight kind oligonucleotides on agarose electrophoresis, it revealed that FQ-1 could intercalate into double-stranded DNA but not single-stranded DNA, the GC contents is needed, and two tandem GC sequence was the minimal requirement for intercalation. Complexes of FQ-1 with 8mer of GC and CG, as double stranded oligonucleotides, were formed in solution and analyzed by electrospray ionization mass spectrometry. A full-scan mass spectra suggests that the specific binding only happened with 5'-d$(GCGCGCGC)_2$-3', supported by the complex ion peak at 1471, along with the helix ion at 1206. Two molecules of FQ-1 were bind on this specific lined 8mer. Our results show that FQ-1 is preferentially a GC sequence selector in 2:1 complexes with ds $(GC)_4$ oligomers.

Keywords: Flouroquinolonyl-ampicillin/ Oligonucleotide/ biding specificity/ antiviral drug/ electrospray ionization mass spectrometry

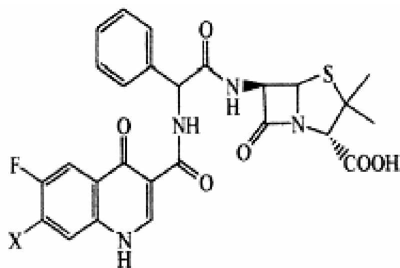
1 Introduction

Drugs interacted with individual bases, nucleosides nucleotides, or the oligonucleotides might reveal their biological activities. Identification of these essential structural elements giving rise to DNA-binding characteristics that may correlate with antitumor activity. By ESI-MS spectrometry, several drugs were successfully in explanation of the intercalation properties into nucleic acid. For noncovalent binding of drug/nucleic acid complexes, it show the specific selection of major or minor groove bindings, or interstrand bindings, binding resulted from electrostatic interactions or interaction of a planar ring system of adjacent base pairs. Further more, characteristic sequences with special selective potentials or tertiary structure of nucleic acids are correlated with drug structures.

In ESI-MS system, molecules in solution can be charged singly or multiply by fine spray in the strong electric field. The charged ions of large biomolecules such as nucleic acid and protein can be identified by their mass-to-charge ratio (m/z), via a series of lenses and travel to the detector, after solving the data from various charged state by calculation of software algorisms [1]. Analysis of noncovalent binding complexes of drugs and large molecules with ESI-MS can provide more detailed information base on the observation on ion peaks corresponding to the complexes with expected molecular mass of complexes and molecules alone. By compilation of these data supplied, can obtain information for assess the binding tendency about groove patterns and bases flanked in particular types. That can be used to evaluate as preclude to DNA cleavage or inhibition of DNA association enzymes [2].

Antitumor drugs such as daunomycin, mithramycin, chromomycin *etc.* were shown to exhibit the interactions with ssDNA, dsDNA, and/or RNA targets [3-6]. By Electrospray ionization Mass Spectrometry (ESI-MS), the drug-nucleic acid complexes formation of noncovalent bound with dsDNA were reported [3-7]. The antibiotic Flouroquinolonyl-ampicillin was the first reported antibiotic synthesized from fluoroquinolone and ampicillin in our lab, in a shortage name as FQ-1. This mosaic antibiotic was reported to possessing the ability of intercalating into double stranded DNA [8]. Its broad anti-infectious spectrum was shown to correlate with the lactam ring structure. Because of the approximate planar structure composed by quinolonyl moiety, benzyl ring and lactam moiety, the intercalating ability into nucleotides have been elucidated and proved with plasmid pUC18 DNA by the mobility shift phenomenon in agarose gel electrophoresis.

The present study was to examine the binding selectivity of this unique mosaic antibiotic to obtain the crucial data about its mechanism of bioactivities.



2 Materials and methods

2.1 Materials

All reagents were analytical grade and MilliQ™ water was used in all experiments. Materials for electrophoresis were products of BIO-RAD. One kb Ladder DNA for MW marker and DNA oligomers for drug complex formation were purchased from New England Bioslabs, Inc. (MA., USA). FQ-1 was prepared in our laboratory as described before, and stocked under -27• in 0.1M. Only one single strand DNA d[5'– GCGCAGCGCAGCGC – 3'] was used; three are formed by repeated base $d[(GC)_7]_2$, $d[(CG)_7]_2$ and $d[(AT)_7]_2$. The remaining four double stranded nucleotides composed by 18, 20, 24 and 29 base pairs.

For ESI-MS spectrometry, four different duplex were studied, all of them are self complementary oligonucleotides d[5'-GCGCGCGC-3' $]_2$, d[5'-CGCGCGCG-3'$]_2$, d[5'-ATATATAT-3'$]_2$ and d[5'-TATATATA-3'$]_2$, these four ds 8mers were stored under -27 • until use.

2.2 Interaction of FQ-1 with DNA

Samples of complex of oligomers and FQ-1, untreated and control standards were applied simultaneously, samples were visualized following electrophoresis using ethidium brimide and UV light observation. The intercalation activities were evaluated by its different behavior of mobility compared with control and untreated samples. In the intercalation reactions monitered by the electrophoretic gel mobility shift assay (EMSA), the reaction mixtures containing 10μl oligomers and 10μl •FQ-1 in different concentration were thoroughly votexed under 37• for 1 hr in 20mM Tris-HCl buffer (pH 8.0) were incubated at 37• for 1 hr in the cases for electrophoresis. Samples of each mixture were analyzed by electrophoresis in 1% agarose, Bands were visualized with ethidium bromide and photographed under UV light. Only four 8mers of dsDNA were studied for ESI-MS. The solutions of oligonucleotide were prepared in 1.0 M ammonium acetate at 0.8mM, annealed by heating to 100• for 10 minutes, then cooling to room temperature about 1-2h. The reaction mixtures of FQ-1 and oligomers were about 4mM after preparation, in 1:1 ratio, reacted under 37• for 1 h. Samples for ESI-MS were diluted four times with 50% methanol ammonium acetate.

2.3 Mass spectrometry

Ten μl aliquots of the samples were injected directly. The carrier liquid was 50% acetonitrile and 0.1% acetic acid as carrier, at a flow rate of 5 μL/min. ESI-MS spectra were obtained with a triple quadrupole mass spectrometer system (Quattro, Micromass, Atrincham, UK). The ESI source was kept at 25•, the capillary was 2.5 kV, the cone voltage was 60 V. The mass scale was calibrated using multiply charged ions standard. Collisional activation was minimized in the quadrapoles. The multiply charged ions formed from the large molecules were detected by monitoring masses in the range of 500 to 2000 Da range (scanned in 5 s). The molecular masses of FQ-1 and oligomers were obtained from the transformed mass spectra; in there the multiply charged ions were calculated and assembled with commercial Mass Lynx transformation program (Micromass, Altrincham, UK).

3 Results

Specific selection of FQ-1 binding with (G/C) rather than (A/T) was observed by Linear double-stranded oligonucleotides composed as repeated GC- or AT- 14-mer oligo pairs was reacted in various molar proportion. FQ-1 at concentration of 125 μg/ml was reacted with (GC) repeated tetrakaidecamer in 156.25 μg/ml, 312.5 μg /ml, 625 μg/ml, 1.25 mg/ml; and 2.5 mg/ml where the relative proportion were calculated as 1 : 2, 1: 1, 2:1, 4:1, and 8:1. Using (AT) repeated tetrakaidecamer in the same set of different concentrations and in both cases drug free solution as control.

3.1 Effect of single strand and double strand

The necessity of double strand for complex forming was examined by using linear

single-stranded and double- stranded GC or CG oligonucleotides. Single-stranded GC- was used as test helix. Fourteen bases of GC- repeated ssDNA was found possessing no binding activity by shift assay. Double- stranded GC or CG oligonucleotides were used to test the 5' end base demand. Fourteen bases of GC-repeated ssDNA alone, and GC or CG repeated double stranded tetrakaidecamer alone were using as control. The binding ability of FQ-1 with each kind of oligomers were tested in the concentration is 312.5 μg/ml. The results were shown that complexes of FQ-1 with 5' GCGCAGCGAGCGC, a non-self complementary DNA exhibited no shift phenomenon. No shift effect was also observed on the complex of 5-$(CG)_7$ oligomer, while 5'-Guanine base 5-$(CG)_7$ duplex/ FQ-1 complex showed significant retardation on EMSA assay. These results reveal FQ-1 is a GC- selective compound.

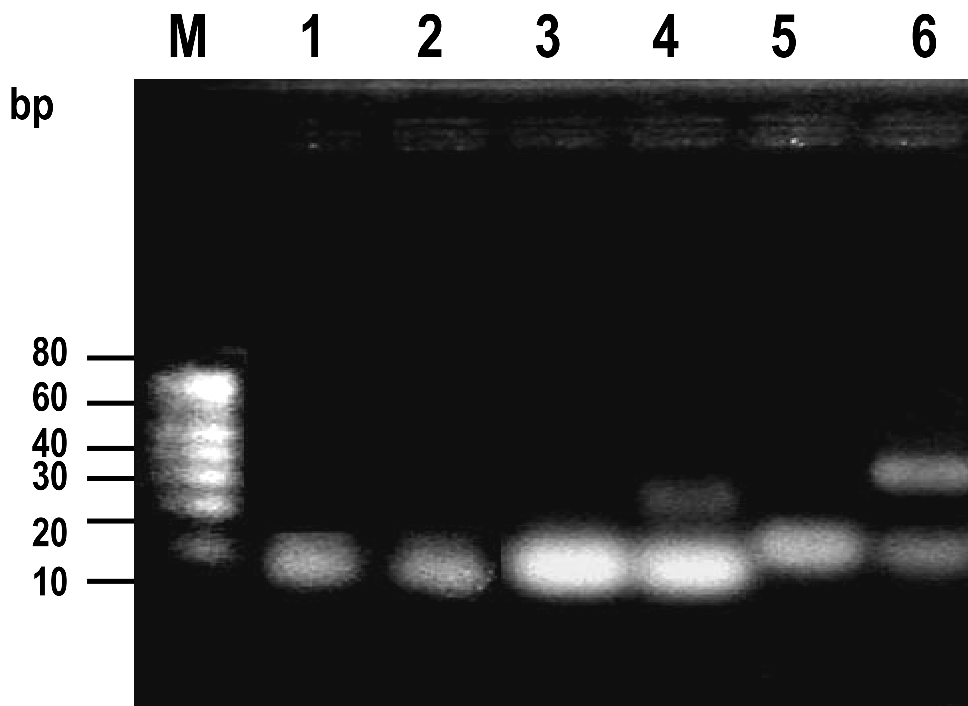


Figure 1. Binding of FQ-1 with linear single-stranded and double- stranded GC or CG oligonucleotides. Single-stranded GC- and double- stranded GC or CG oligonucleotides were used as tested DNA. Lanes M: 10 bp DNA marker; Lanes 1: ss DNA alone; Lane 2: ss DNA+ FQ-1; Lane 3: ds CG DNA alone; Lane 4: ds CG DNA + FQ-1; Lane 5: ds GC DNA alone; Lane 6: ds GC DNA + FQ-1. The concentration of FQ-1 is 312.5 μg/ml. ss DNA：single-stranded DNA; ds DNA：double-stranded DNA.

3.3 Electrospray ionization MS Spectrometry

By assuming that relative intensity observed by ESI MS reflect the relative abundance in solution, the complex ion was observed in a low proportion. On ESI-MS spectra the reaction results of 2:1 FQ-1 derivatives and 5'-d$(GC)_4$-3' reaction mixture were evaluated. There are three groups of ion species composed by drug/DNA complexes were observed; two kinds of oligomers assigned as duplex around m/z 1606, and mixture of simplex and duplex around m/z1204 with zero to three sodium ions. Three derivatives of FQ-1 were existed in the mixture, as negative ion peaks m/z 512, 537 and 557 respectively. At least four kinds of drug dimmers can be recognized from m/z1000~m/z1100.

There existed complexes of FQ-1 derivatives/simplex and FQ-1 derivatives/simplex. Suspected FQ-1/duplex complexes were assigned as ions corresponding to m/z 1793. ($[2M+I_1-2H]^{2-}$, K),

FQ-1/simplex were assigned to m/z 1471. ($[M+I_1-2H]^{2-}$, G). Intercalates of duplex with two drugs molecules were observed also around m/z 1998 ($[M+I_1-2H]^{2-}$, N). Other drug derivatives as m/z 512 and m/z 557 were observed to form complexes in the same mode as FQ-1, assigned as m/z 1462 ($[M+I_2-2H]^{2-}$, F), m/z 1787 ($[2M+I_2-2H]^{2-}$, L) and m/z 1972 ($[2M+2I_2-2H]^{2-}$, M).

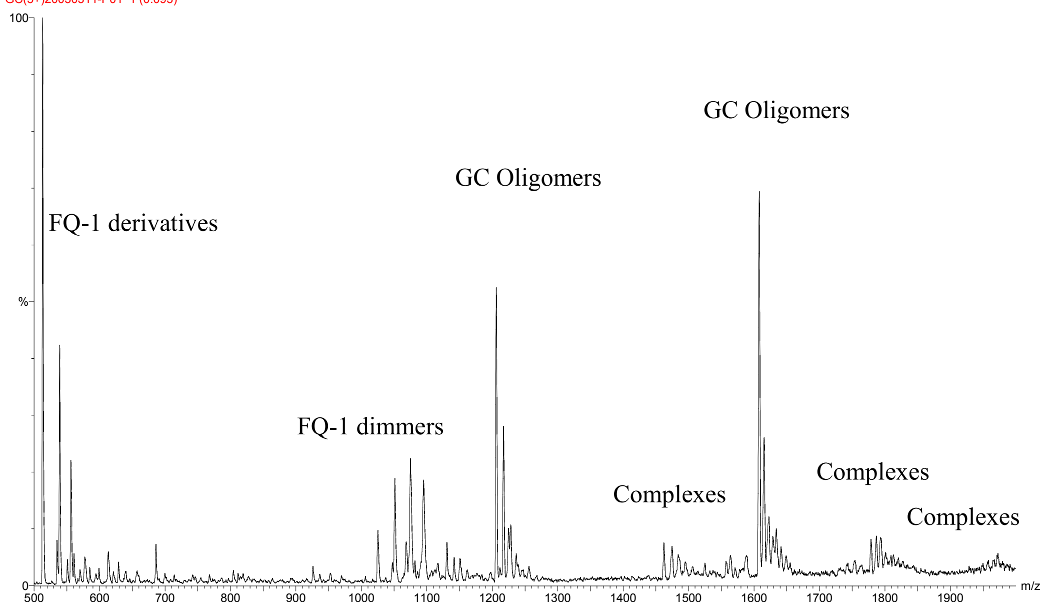


Figure 2. Full scan of ESI mass spectra mixtures of FQ-1 derivatives in intercalation experiment on 5'-d(GCGCGCGC)-3'. Three derivatives of FQ-1 and four kinds of dimmers were observed. Oligomers were assigned as duplex and simplex, three groups of complexes were found.

4 Discussion

Complexes observed in ESI MS experiments, were maintained in the high concentration of volatile stabilizing counter ions, nonspecific electrostatic association of FQ-1 derivatives with DNA was minimized. Because of the extreme low concentration of reaction molecules, electrostatic aggregation was also excluded. The nonspecific binding was also excluded by the experiment using mixtures of drugs and DNA without 37• incubation period.

In these experiments, self-complementary oligonucleotides were annealed to form duplex DNA, the self reannealed simplex oligomer and its drug-intercalated complexes cannot be ruled out from mass spectra. Because the results of electrophoresis suggested no complex formed in simplex case, and no complex formed from the results of samples preheating at 37• and 80• cause much higher intensity of ions corresponding to $[M-2H]^{2-}$/ $[2M-4H]^{4-}$. Samples formed by the oligomers lasted with 100• preheating and cooling down, cause a enhancing effect of ions corresponding to $[2M-3H]^{3-}$ and complexes also appeared. These results confirmed the demand of the duplex for intercalation of FQ-1 derivatives. The intensity of the number of bound drug molecules exhibited no discrepancy with this point. The intercalates of nogalamycin and daunomycin show the relative high intensity located in the 3~4 drug molecules, and seldom one drug complexes formed, there exhibited no expelling of simplex intercalates can be confirmed by MS experiments. But in this study the intensity of one drug molecule complexes were rather higher than two drug molecules

complexes. The flexibility of the rotation along the covalent bonds between the rings and the hydrogen bonding for the stabilization of larger planar structure caused a rugged planar structure, that might cause the intercalation reaction rather difficult than other intercalating agents.

The relative intensity of the complexes caused by threeFQ-1 derivatives in ESI mass spectra, showed the similar relative intensity of each individual drugs. These results revealed that the side chain donates no effect on intercalation reactions. The main cause of complexes forming is the ring systems. Conditions suitable for the stability of larger planar forms exist, can promote the reaction happens. The intercalating ability might shift in electric field by electrophoretic gel mobility shift assay (EMSA), but ESI-MS can supply more detail information. Minor groove binders have been donated to possessing high affinity with at least four consecutive A or T; otherwise, positive charge, many proton donor or receptor groups. The flat aromatic part of FQ-1 may make it to stack between the base pairs of the duplex. The lack of positive charge and donor may make it hard to fix to improve these the EMSA was used to differentiate the preferences, and then select a series of designed oligomers to define the minimum need for the binding then proved by ESI-MS.

There are seldom reports on the specific orientation happened with the 5' end GC, or 5' end CG, this study shown that FQ-1 is preferentially a GC sequence selector in 2:1 complexes with ds $(GC)_4$ oligomers and its orientation specificity deserve further study about the three dimension topology of its relation with stoichiometry.

5 Concluding remarks

FQ-1 could intercalate into double-stranded DNA but not single-stranded DNA, the GC contents is needed, and two tandems GC sequence was the minimal requirement for intercalation. No complex forms from the results of AT oligomers experiments, not only the migration shifts on agarose electrophoresis and ion peaks in ESI mass spectra. Complexes formed of FQ-1 derivatives with 8mer of GC and CG, as double stranded oligonucleotides, also suggested that the specific binding only happened with 5'-dGCGCGCGC-3', corresponding to the ion peaks at 1471, along with the helix ion at 1206. Two molecules of FQ-1 or its derivatives were binding on this specific lined 8mer. We concluded that FQ-1 and its derivatives are preferentially a GC sequence selectors in 2:1 complexes with ds $(GC)_4$ oligomers.

5 References

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