Supporting Information

Renal Protein Reactivity and Stability of Antibiotic Amphenicols: Structure and Affinity

Fei Ding,[†abc] Wei Peng,*[†b] Yu-Kui Peng*[a] and Yu-Ting Jiang[d]

[a] *College of Food Science & Engineering, Northwest A&F University, Yangling 712100, China*

[b] *Department of Chemistry, China Agricultural University, Beijing 100193, China*

[c] *Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States*

[d] *Department of Chemistry, University of Ottawa, 10 Marie Curie, Ottawa, ON K1N 6N5, Canada*

[†]These authors contributed equally to this work.

*Corresponding Author

E-mail: pyk2009@nwsuaf.edu.cn (Y.-K. Peng). Phone/fax: +86-29-87092367.

E-mail: jurryw.peng@gmail.com, weipeng@cau.edu.cn (W. Peng). Phone/fax: +86-10-62734195.

Time-Resolved Fluorescence. Time-resolved fluorescence was acquired with a FLS920 spectrometer (Edinburgh Instruments, UK), using the time-correlated single-photon counting system with a hydrogen flash lamp excitation source, in air-equilibrated solution at ambient temperature. The excitation wavelength was 295 nm, and the number of counts gathered in the channel of maximum intensity was 4,000. The instrument response function (IRF) was gauged exploiting Ludox to scatter light at the excitation wavelength. The data were analyzed with a nonlinear least-squares iterative method utilizing the Fluorescence Analysis Software Technology, which is a sophisticated software package designed by Edinburgh Photonics for the analysis of fluorescence and phosphorescence decay kinetics, IRF was deconvoluted from the experimental data, and the resolution limit after deconvolution was 0.2 ns. The value of χ^2 (0.9～1.2), the Durbin-Watson parameter (greater than 1.7) as well as a visual inspection of the residuals were used to evaluate how well the calculated decay fit the data. Average fluorescence lifetime (τ) for multiexponential function fittings were from the following relation:[1]

$$I(t) = \sum_i A_i e^{\frac{-t}{\tau_i}} \tag{1}$$

where τ_i are fluorescence lifetimes and A_i are their relative amplitudes, with i variable from 1 to 2.

Circular Dichroism. Far-UV CD spectra were collected with a Jasco-815 spectropolarimeter (Jasco, Japan), equipped with a microcomputer, the instrument was sufficiently purged with 99.9% dry nitrogen gas before starting the apparatus, and then it was calibrated with d-10-camphorsulfonic acid. All of the CD measurements were picked at 298 K with a PFD-425S Peltier temperature controller attached to a water bath with an accuracy of ±0.1 ^{o}C. Each spectrum was scanned with the use of quartz cuvette of 0.2 cm path length and taken at wavelength between 200 and 260 nm with 0.1 nm step resolution and averaged over five scans operated at a speed of 20 nm min^{-1} and response time of 1 s. A reference sample containing buffer and chloramphenicol was subtracted from the CD signal for assay, and the secondary structure was appraised exploiting Jasco Spectra Manager II, which computes the different designations of secondary structures by comparison with raw CD spectra, determined from distinct proteins for which high-quality X-ray diffraction data are available.[2-4]

Theory of Fluorescence Quenching. Quenching of fluorescence is described by the Stern-Volmer equation:[1,5]

$$\frac{F_0}{F} = 1 + k_q[Q] = 1 + K_{SV}[Q] \quad (2)$$

In this equation F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively; k_q is the bimolecular quenching constant; τ_0 is the lifetime of the fluorophore in the absence of quencher, [Q] is the concentration of quencher, and K_{SV} is the Stern-Volmer quenching constant. Therefore equation (2) was used to estimate K_{SV} by linear regression of a plot of F_0/F *versus* [Q].

Calculation of Reactive Ability. When ligand molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound ligand molecules is given by the relationship:[6-8]

$$\log\frac{F_0 - F}{F} = \log K + n\log[Q] \tag{3}$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively; K and n are the association constant and the kind of binding sites, respectively; and [Q] is the concentration of quencher. Thus, a plot of log(F_0—F)/F *against* log[Q] can be used to assess K and n. The fluorescence intensities were corrected for absorption of the exciting light and reabsorption of the emitted light to decrease the inner filter effect using the equation:[1]

$$F_{cor} = F_{obs} \times e^{\frac{A_{ex} + A_{em}}{2}} \tag{4}$$

In this equation F_{cor} and F_{obs} are the fluorescence intensities corrected and observed, respectively; and A_{ex} and A_{em} are the absorption of the systems at the excitation and emission wavelengths, respectively. The fluorescence intensity used in this study is the corrected intensity.

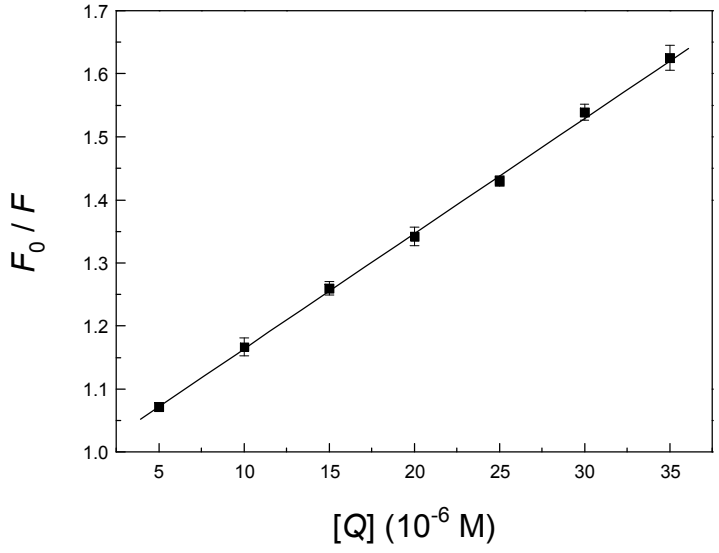

Fig. S1. Stern-Volmer plot narrating lysozyme Trp quenching at pH＝7.4 and T＝298 K induced by chloramphenicol association. y＝0.01839x＋0.9803, R＝0.9996, based on equation (2). Fluorescence emission intensity was registered at λ_{ex}＝295 nm, and the λ_{em} maximum emerged at 337 nm. All data were corrected for quencher fluorescence and each data was the average of three individual experiments±S.D. ranging 0.64%－1.96%.

Table S1

Fluorescence lifetime of lysozyme as a function of concentrations of chloramphenicol

c(chloramphenicol)	τ_1 (ns)	τ_2 (ns)	A_1	A_2	τ (ns)	χ^2
0	1.39	3.41	0.77	0.23	1.85	0.99
10	1.33	3.39	0.75	0.25	1.85	1.01
20	1.29	3.43	0.76	0.24	1.80	1.02
40	1.25	3.14	0.69	0.31	1.84	0.98
80	1.23	3.29	0.71	0.29	1.83	1.02

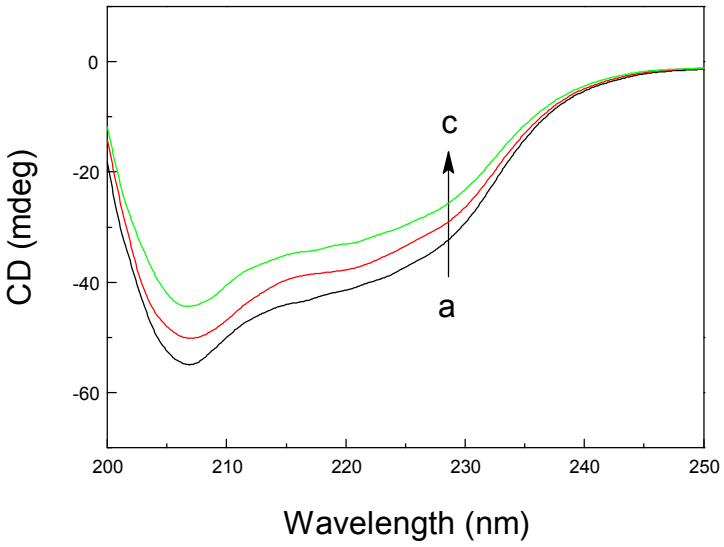

Fig. S2. Far-UV CD spectra of lysozyme complexes with chloramphenicol (pH＝7.4, T＝298 K). (a) 4.0 μM lysozyme; (b) 4.0 μM lysozyme＋12 μM chloramphenicol; (c) 4.0 μM lysozyme＋24 μM chloramphenicol.

Table S2

Secondary structure components of lysozyme complexes with chloramphenicol at pH＝7.4 assessed by Jasco Spectra Manager II Software

Samples	Secondary structure components (%)			
	α-helix	β-sheet	Turn	Random
Free lysozyme	42.1	17.6	14.4	25.9
Lysozyme＋chloramphenicol (1∶3)	38.8	18.4	15.7	27.1
Lysozyme＋chloramphenicol (1∶6)	34.3	19.3	17.2	29.2

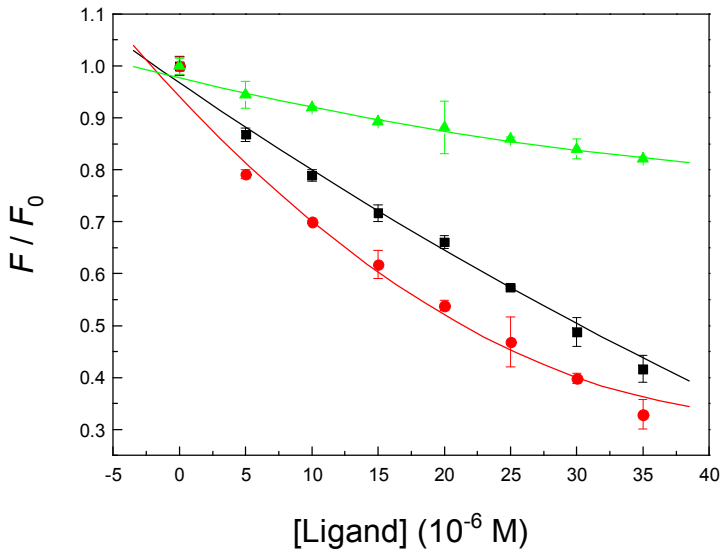

Fig. S3. Fluorescence quenching patterns of lysozyme and ANS-lysozyme mixture at pH＝7.4, *T*＝298 K. Binding isotherm of chloramphenicol (■) and ANS (●) aroused quenching of lysozyme Trp fluorescence and quenching of ANS-lysozyme adduct fluorescence by chloramphenicol (▲). All data were corrected for quencher fluorescence. Each value was the mean of three separate measurements±S.D. ranging 0.27%－5.01%.

ABBREVIATIONS USED

ANOVA, analysis of variance; ANS, 8-anilino-1-naphthalenesulfonic acid; Arg, arginine; Asp, aspartic acid; CD, circular dichroism; Cys, cysteine; His, histidine; Ile, isoleucine; IRF, instrument response function; Leu, leucine; LMW, low-molecular-weight; NAG, *N*-acetyl-glucosamine; NAM, *N*-acetylmuramic acid; R, correlation coefficient; S.D., standard deviation; Thr, threonine; Tris, tris(hydroxymethyl)aminomethane; Trp, tryptophan; UV/vis, ultraviolet-visible spectroscopy.

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