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MUCIN BINDING TO THERAPEUTIC MOLECULES: THE CASE OF ANTIMICROBIAL AGENTS USED IN CYSTIC FIBROSIS

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Abstract

Mucin is a complex glycoprotein consisting of a wide variety of functional groups that can interact with exogenous agents. The binding to mucin plays a crucial role in drug pharmacokinetics especially in diseases, such as cystic fibrosis (CF), where mucin is overexpressed. In this study, we have investigated the interaction between mucin and several drugs used in CF therapy. Protein-drug interaction was carried out by UV-Vis and fluorescence spectroscopy; quenching mechanism, binding constants, number of binding sites, thermodynamic parameters and binding distance of the interaction were obtained.

Keywords: mucin, antibiotics, cystic fibrosis, UV-Vis, fluorescence.

1. INTRODUCTION

Many epithelial surfaces in the human body are covered with a thin mucus layer. The airways, the entire gastrointestinal tract, and the eyes are only few of the sites where mucus can be found. Structurally, the mucus is a semi-permeable viscoelastic hydrogel with heterogeneous composition. Indeed, it consists of about 95% by water and approximately 5% by electrolytes, lipids, fragments of DNA (especially observed in pathological states) and different types of proteins (Creeth, 1978; Murgia et al., 2017). By acting as a barrier, mucus primary function is to protect the underlying epithelium from noxious agents, such as air pollutants or bacteria. The defensive activity of mucus can be principally attributed to mucins, that are the primarily expressed proteins within mucus.

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Mucins are described as long polymeric glycoconjugates having high molecular weight ($1-40 \times 10^6$ Da, (Taherali et al., 2017)), consisting of a linear peptide backbone, rich in tandem repeats of proline, threonine and serine (PTS domains). To PTS domains a huge amount of carbohydrate chains (up to 40-80% by weight) are O-linked (García-Díaz et al., 2017; Johansson et al., 2011). The bounds are formed between hydroxylic groups on the peptide backbone, with fragments of fucose, galactose, sialic acids, *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) on the polysaccharide chains (Boegh and Nielsen, 2015). Also present, but less abundant, are the N-linked glycans to the N-terminus. The glycosylated and highly hydrophilic domains are separated by “naked” cystine rich and more hydrophobic domains that fold into globules stabilized by multiple internal disulfide bonds (Cone, 2009). In all the secretory mucins, domains similar to the von Willebrand Factor (vWF) C and D are present at the N-terminal, and likewise, at the C-terminal cysteine knot domains are exposed. Mucins exhibit an overall negative charge due to the free carboxylate (sialic acid) and sulfonate groups present in the glycosylated PTS domains. The overall negative charge increases the stability of the mucin network thanks to repulsive forces.

Since the mucin-drug interaction may have an important role on drug pharmacokinetics (as a strong bind with the protein may result in a reduced drug absorption, hence a low drug efficacy), in this study we have investigated the interaction between several drugs of interest in cystic fibrosis (CF) and

mucin (Figure 2). Since CF patients are susceptible to concurrent chronic pulmonary infections by bacteria, which gradually destroys lung tissues, one of the major goals of CF therapies involves the maintenance of the pulmonary function (“Cystic Fibrosis Foundation [US],” 2019). With this purpose, antibiotics are a standard of care for CF patients. Anti-infective therapies are employed to prevent, eradicate and control respiratory infections. By fighting bacterial infections, the antibiotic treatments lead to a reduction of the inflammatory milieu, therefore an improvement of the pulmonary function. One of the most recurrent and aggressive bacteria is *Pseudomonas aeruginosa*. If not detected and treated adequately, infections related to this gram-negative, opportunistic bacterium become chronic, which resultant inflammatory response is closely linked to decreased pulmonary function (Edmondson and Davies, 2016). While *P. aeruginosa* is the most aggressive bacteria for CF patients, other pathogens as *Staphylococcus aureus* or *Burkholderia cepacia* can also infect the airways, therefore different classes of antibiotics are employed in CF therapy. Inhaled tobramycin associated with aztreonam lysine is usually the first line of treatment for early infections.

The affinity of some drugs for mucin has been previously studied using spectroscopic methods by Pontremoli *et al* (Pontremoli et al., 2015), and by chromatographic methods by Gargano *et al* (Gargano et al., 2014), whereas Barbero *et al* have investigated the interactions of both carbon nanotubes (CNTs) and gold nanoparticles (GNPs) with mucin (Barbero et al., 2018, 2016). Yet, much needs still to be uncover about mucin-drugs interaction and the physico-chemical parameters that govern the affinity of drugs for mucin. In this work, antibiotics from different anti-infective classes were employed to assess their interaction with mucin, including ceftazidime, and its core chemical structure, 7-aminocephalosporanic acid (7-ACA), aztreonam, ampicillin, tobramycin, levofloxacin and rifampicin. Moreover, a selective and reversible inhibitor of the transmembrane protein CFTR (CFTR(inh)-172) has been tested too (Thiagarajah et al., 2004).

Figure 2. Chemical structures and pharmaceutical class of the tested molecules

2. MATERIALS AND METHODS

2.1 Materials

Mucin from porcine stomach (PGM type III, bound sialic acid 0.5-1.5%, partially purified powder), aztreonam, CFTR(inh)-172, ampicillin, tobramycin, levofloxacin, 7-ACA and rifampicin were purchased from Sigma Aldrich. Commercial ceftazidime for injection was supplied from Fresenius Kabi. PGM and drugs stock solutions for UV and fluorescence analysis were prepared in PBS (phosphate buffer solution 2 mM, pH = 7.4). Dissolution of the powder compounds was enhanced by sonication in an ultrasonic bath. The CFTR(inh)-172 stock solution was prepared by previously dissolution of the powder into a minimum volume of EtOH. All chemicals were of analytical reagent grade and were used without further purification. Millipore grade water was obtained from an in-house Millipore system (resistivity: 18.2 M Ω cm at 25 °C).

2.2 Methods

2.2.1 UV-Vis absorption spectroscopy

UV-Vis absorption spectra were measured by a UH5300 Hitachi spectrophotometer at room temperature, using quartz cuvettes (1 cm pathway length). The UV measurements were made in the range of 200-400 nm (with the exception of CFTR(inh)-172 where the range was set on 200-500 nm). Spectra of a 0.05 mg/mL mucin solution were recorded in the absence and in the presence of increasing concentrations of drugs (Figure 3. C_{7-ACA} (a-g) = 0, 5, 10, 15, 20, 25, 30 μ M; $C_{ceftazidime}$ (a-f) = 0, 5, 10, 20, 40, 60 μ M; $C_{aztreonam}$ (a-g) = 0, 5, 10, 15, 20, 30, 40, 50 μ M; $C_{ampicillin}$ (a-g) = 0, 25, 50, 100, 200, 500, 800 μ M; $C_{CFTR(inh)-172}$ (a-g) = 0, 2, 5, 7, 10, 15, 20 μ M; $C_{tobramycin}$ (a-h) = 0, 25, 50, 100, 200, 500, 800, 1600 μ M; $C_{levofloxacin}$ (a-g) = 0, 1, 2, 3, 4, 6, 8 μ M; $C_{rifampicin}$ (a-g) = 0, 1, 2, 4, 8, 12, 20 μ M.).

2.2.2 Emission spectroscopy

Fluorescence emission spectra in steady state mode were acquired at three temperatures (296, 303 and 310 K) using a Horiba Jobin Yvon Fluorolog 3 TCSPC fluorimeter equipped with a 450-W Xenon lamp and a Hamamatsu R928 photomultiplier.

Fluorescence spectra were recorded in the range of 290-480 nm and the excitation wavelength was fixed on 280 nm. Excitation and emission slit width was set on 6 and 7, respectively. A constant concentration (0.05 mg/mL) of PGM was analysed by successive increasing the concentration of the drugs (Figure 4. C_{7-ACA} (a-l) = 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 μ M; $C_{ceftazidime}$ (a-i) = 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6 μ M; $C_{aztreonam}$ (a-i) = 0, 0.1, 0.5, 1, 2, 3, 4, 5, 6 μ M; $C_{ampicillin}$ (a-k) = 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 μ M; $C_{CFTR(inh)-172}$ (a-h) = 0, 0.05, 0.1, 1, 1.5, 2, 2.5, 3 μ M; $C_{tobramycin}$ (a-i) = 0, 150, 200, 500, 600, 800, 1000, 1200, 1600 μ M; $C_{levofloxacin}$ (a-j) = 0, 0.005, 0.01, 0.09, 0.15, 0.25, 0.5, 1, 1.5, 2 μ M; $C_{rifampicin}$ (a-i) = 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 μ M.). After preparation, samples were left to equilibrate for at least 30 min and only then fluorescence measures were performed.

To minimize the inner filter effect (IFE), the concentration of the different drugs was set in order to have absorbance values lower than 0.1. However, as reported by Arumugam *et al.*, a change in

absorption equal to 0.03 corresponds to a 3% reduction in emission intensity of protein, so it is necessary to correct the observed emission intensity for IFE even for absorption values lower than 0.1 (Sharma et al., 2014). The IFE correction was done for the fluorescence intensities of mucin and drug using the following equation:

$$F_{corrected} = F_{observed} \cdot 10^{\frac{(A_{ex}+A_{em})}{2}} \quad (1)$$

where $F_{corrected}$ and $F_{observed}$ are the corrected and observed fluorescence intensities, respectively. A_{ex} and A_{em} are the solution absorbance at the excitation and emission wavelengths, respectively.

3. RESULTS AND DISCUSSION

3.1 UV-Vis absorption studies

UV-Vis absorption spectroscopy is a simple and commonly employed technique for studying the interactions between proteins and small molecules (Sirajuddin et al., 2013; Zhang et al., 2008). By monitoring the changes in the absorption properties of the protein, it is possible to detect the formation of a complex between the protein and the small molecule. It has been assumed that a shift of the maximum peak of the protein in the presence of the ligand is a proof of the formation of a complex between the two species. Mucin has an absorption peak at 257 nm principally due to the presence of the amino acid phenylalanine.

The UV-Vis absorption spectra of mucin were recorded by keeping constant the concentration of protein, while the concentration of drug was varied increasingly (Figure 3). Except for tobramycin (Figure 3-F), that have no effect at all upon mucin, the UV absorption intensity was gradually enhanced for PGM following the addition of increasing amounts of drug (hyperchromism). These changes may be explained by the formation of a new mucin-drug complex.

3.2 Steady state fluorescence spectroscopy

Fluorescence spectroscopy is probably one of the most commonly technique used to study interaction between small molecules and proteins. Normally, the fluorescence of proteins is caused only by three intrinsic fluorophores present in the protein, i.e. tryptophan, phenylalanine and tyrosine amino acids (Sirajuddin et al., 2013; Zhang et al., 2008). After excitation at 280 nm, PGM shows a strong fluorescence emission at 350 nm due to the presence of tryptophan residues.

Since tryptophan fluorescence intensity is extremely sensitive to the local environment, in the present work, the interaction of drugs to PGM was evaluated by measuring the intrinsic fluorescence of the protein before and after addition of increasing concentrations of drug. It was observed that the fluorescence intensities of PGM was gradually reduced with increasing concentrations of ceftazidime, aztreonam, CFTR(inh)-172, and levofloxacin (Figure 4, graphs B, C, E, and G, respectively). It is possible to assume that during the binding interaction, the aforementioned drugs have altered tryptophan microenvironment. No significative variations of fluorescence intensities were detected in the presence of 7-ACA, ampicillin, tobramycin, and rifampicin (Figure 4, graphs A, D, F, and H, respectively). The process that decreases the intensity of the fluorescence emission is known as fluorescence quenching. Several processes can result in quenching, such as excited state reactions, energy transfer, ground-state complex formation (static quenching) and collisional quenching (also called dynamic quenching). Both static and dynamic quenching require molecular contact between the

fluorophore (mucin) and the quencher (drug). In the case of dynamic quenching, the quencher diffuses to the fluorophore and, upon contact, the fluorophore returns to the ground state without emission of a photon. On the other hand, in static quenching a non-fluorescent complex is formed between the fluorophore and the quencher (Lakowicz, 2006).

Figure 3. UV-Vis absorption spectra of mucin (PGM, blue line) in the presence of 7-ACA (A), ceftazidime (B), aztreonam (C), ampicillin (D), CFTR(inh)-172 (E), tobramycin (F), levofloxacin (G), and rifampicin (H). The red dashed lines reports the absorption spectra of the drugs alone at a concentration that gives an absorbance of about 0.1 (7-ACA 12 μ M, ceftazidime 6 μ M, aztreonam 10 μ M, ampicillin 500 μ M, CFTR(inh)-172 5 μ M, tobramycin 1600 μ M, levofloxacin 2 μ M, and rifampicin 5 μ M).

Figure 4. Steady state fluorescence spectra of mucin (PGM) in the presence of 7-ACA (A), ceftazidime (B), aztreonam (C), ampicillin (D), CFTR(inh)-172 (E), tobramycin (F), levofloxacin (G), and rifampicin (H). The red dashed lines report the fluorescence emission spectra of drug alone at the maximum concentration used.

3.2.1 Quenching mechanism of PGM in the presence of drugs

The fluorescence quenching data were analysed according to Stern-Volmer equation:

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

Where F_0 is the fluorescence intensity of mucin alone and F is the fluorescence of mucin in the presence of increasing concentrations of quencher $[Q]$. K_{SV} is the Stern-Volmer quenching constant. $[Q]$ is the quencher (drug) concentration. Quenching data are presented as plots of F_0/F vs $[Q]$, yielding an intercept of one on the y axis and a slope equal to K_{SV} (Figure 5).

Figure 5. Stern-Volmer plots of PGM - ceftazidime (B), PGM - aztreonam (C), PGM - CFTR(inh)-172 (E), and PGM – Levofloxacin (G).

Linear Stern-Volmer plots does not prove that collisional quenching of fluorescence has occurred. Static and dynamic quenching can be distinguished by their differing dependence on temperature. Larger amounts of collisional quenching are observed at higher temperatures due to the faster diffusion rates, while smaller amounts of static quenching are observed at higher temperatures since less weakly bound complexes are formed (Lakowicz, 2006).

The K_{SV} of PGM – ceftazidime and PGM – CFTR(inh)-172 increases with the increasing of temperature (checked at 296 K, 303 K and 310 K. See Table 2), which implies a dynamic quenching between mucin and the aforementioned drugs. The K_{SV} of PGM – aztreonam and PGM – levofloxacin is similar with negligible variations upon increasing temperature.

Table 2. The quenching constants of mucin (PGM) and drugs at different temperatures.

	$K_{SV} (M^{-1}) 10^4$		
	296 K	303 K	310 K
<i>Ceftazidime</i>	2,5 (± 0.17)	3,7 (± 0.30)	7,0 (± 0.36)
<i>Aztreonam</i>	2,6 (± 0.17)	2,3 (± 0.15)	2,5 (± 0.09)
<i>CFTR(inh)-172</i>	5,4 (± 0.16)	6,1 (± 0.19)	6,9 (± 0.24)
<i>Levofloxacin</i>	4,3 (± 0.20)	4,0 (± 0.16)	5,1 (± 0.46)

3.2.2 Binding parameters

For the determination of the association constant (K_A) and the number of binding sites (n), the double logarithm regression curve (Equation 3) was used.

$$\log \frac{(F_0 - F)}{F} = \log K_A + n \log [Q] \quad (3)$$

where F_0 and F are the fluorescence intensities in absence and presence of quencher (drug), K_A denotes the binding constant, n refers to the number of binding sites on the protein, and $[Q]$ is the quencher concentration. Dissociation constant (K_D) was calculated as the reciprocal of K_A . From the plot of $\log[(F_0 - F)/F]$ vs $\log[Q]$ it is possible to calculate n and $\log K_A$ as it represents the slope of the line, and its interception on the y axis, respectively. Values of n , K_A and K_D are reported in Table 3.

Based on the values of n almost equal to 1, one binding site on mucin for each drug can be deduced. It was found that the values of K_A for PGM – ceftazidime and PGM – CFTR(inh)-172 interactions increased with a rise in temperature, which implies a dynamic quenching. While, for PGM – aztreonam and PGM – levofloxacin interactions, the rise in temperature does not affect significantly the K_A . The overall low values of K_A reflect the establishment of a weak bound with mucin.

Table 3: values of the number of binding sites on mucin for each mucin-drug complex (n), association (K_A) and dissociation constant (K_D).

	n	$K_A (M^{-1}) 10^3$	$K_D (M) 10^{-4}$
<i>Ceftazidime</i>			
296 K	0.74 (± 0.040)	1.1 (± 0.081)	9.14
303 K	0.73 (± 0.064)	1.3 (± 0.15)	7.62
310 K	0.78 (± 0.039)	4.6 (± 0.29)	2.16
<i>Aztreonam</i>			
296 K	0.64 (± 0.035)	0.32 (± 0.026)	32
303 K	0.63 (± 0.064)	0.26 (± 0.038)	39
310 K	0.64 (± 0.11)	0.30 (± 0.076)	34
<i>CFTR(inh)-172</i>			
296 K	0.72 (± 0.049)	1.3 (± 0.12)	7.8
303 K	0.81 (± 0.032)	5.0 (± 0.25)	2.0
310 K	0.93 (± 0.045)	24 (± 1.5)	0.41
<i>Levofloxacin</i>			
296 K	0.75 (± 0.049)	1.4 (± 0.14)	6.9

303 K	0.90 ($\pm$ 0.098)	9.4 ($\pm$ 1.4)	1.1
310 K	0.72 ($\pm$ 0.0094)	0.96 ($\pm$ 0.019)	10

3.2.3 Thermodynamic parameters

A ligand can bind a protein basically through four types of non-covalent interactions: van der Waals forces, hydrogen bond, hydrophobic and electrostatic interactions. The thermodynamic parameters, enthalpy (ΔH°) and entropy (ΔS°), are used as evidence for the nature of the acting forces (Chaves et al., 2017). According to the data of enthalpy and entropy changes, the model of protein-drug interaction can be summarized as (Pontremoli et al., 2015): (i) $\Delta H^\circ > 0$ and $\Delta S^\circ > 0$ are indicative of binding driven by hydrophobic forces; (ii) $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$ interactions through van der Waals interactions and hydrogen bonds; (iii) $\Delta H^\circ < 0$ and $\Delta S^\circ > 0$ are indicative of electrostatic interactions. The thermodynamic parameters were calculated through Van't Hoff equations:

$$\ln K_A = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (4)$$

$$\Delta G^\circ = -RT \ln K_A \quad (5)$$

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \quad (6)$$

where K_A is the binding constant, R is the gas constant ($8.31447 \text{ J mol}^{-1} \text{ K}^{-1}$), T are the experimental temperatures (296 K, 303 K, 310 K) and ΔG° corresponds to the Gibbs free energy variation. Values of ΔH° , ΔS° and ΔG° are summarized in Table 4. As it can be observed from Table 4, for mucin – ceftazidime, mucin – CFTR(inh)-172 and mucin – levofloxacin complexes both ΔH° and ΔS° are positive, therefore hydrophobic interactions are the predominant driving forces for the formation of these protein-drug complexes. On the other hand, a positive and negative value of ΔH° and ΔS° respectively, have been found for mucin – aztreonam complex, so this implies that mostly electrostatic forces occur between mucin and drug. The binding process is assumed to be spontaneous as ΔG° values are negative for all interactions.

Table 4. Thermodynamic parameters for mucin-drug complexes at different temperatures

	$\Delta G \text{ (kJ mol}^{-1}\text{)}$	$\Delta H^\circ \text{ (kJ mol}^{-1}\text{)}$	$\Delta S^\circ \text{ (J mol}^{-1} \text{ K}^{-1}\text{)}$
<i>Ceftazidime</i>			
296 K	-24.92		
303 K	-26.47	56.22	273.7
310 K	-28.76		
<i>Aztreonam</i>			
296 K	-25.04		
303 K	-25.32	-0.74	81.77
310 K	-26.20		
<i>CFTR(inh)-172</i>			
296 K	-26.82		
303 K	-27.75	13.66	136.7

310 K	-28.73		
<i>Levofloxacin</i>			
296 K	-26.28		
303 K	-26.69	8.76	117.9
310 K	-27.94		

It seems that an undefined relation exists between drug lipophilicity and interaction with mucin, particularly, hydrophobic drugs tend to interact more with mucin. As reported in Figure 6, the greater the $\log D_{7.4}$ is, the greater the K_{SV} . Indeed, CFTR(inh)-172 that is the more lipophilic molecule at pH 7.4 has the greater value of K_{SV} , followed by levofloxacin with a lower value of $\log D_{7.4}$; aztreonam and ceftazidime, which are the more hydrophilic drugs at pH 7.4, have the lowest values of K_{SV} .

Figure 6. Histogram of the Stern-Volmer constants (K_{SV}) and values of $\log D_{7.4}$ of drugs. Values of $\log D_{7.4}$ are predicted by means of ChemAxon.

3.2.4 Resonance energy transfer

One important process that can occur in the excited state is the resonance energy transfer, RET (also called fluorescence, or Förster, energy transfer, FRET). This process takes place whenever the emission spectrum of a fluorophore, called the donor, overlaps with the absorption spectrum of another molecule, called the acceptor. The distance between donor and acceptor, as well as the extent of spectral overlap, determine the amount of energy transfer (Lakowicz, 2006). The efficiency of energy transfer can be calculated according to Equation 7:

$$E = 1 - \left(\frac{F}{F_0} \right) = \frac{R_0^6}{R_0^6 + r^6} \quad (7)$$

where E can be determined experimentally from the donor (mucin) emission in the absence (F_0) and presence of acceptor (F); r is the distance between the donor and the acceptor (drug); R_0 is the critical distance when the efficiency of transfer is 50%. R_0 can be obtained with the Equation 8:

$$R_0^6 = 8.79 * 10^{-5} [k^2 n^4 \varphi J(\lambda)] \quad (8)$$

where k^2 is the orientation factor, n is the refractive index of the medium, φ is the fluorescence quantum yield of the donor, and J is the overlap integral between the fluorescence emission spectrum of donor and the absorption spectrum of the acceptor, and it can be obtained according to Equation 9:

$$J = \frac{F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (9)$$

where $F(\lambda)$ is the fluorescence intensity of the donor at wavelength λ and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength λ . In this case $k^2 = 2/3$, $n = 1.336$ and $\varphi = 0.118$ (Pontremoli et al., 2015).

An example, the overlaps of emission spectra of mucin and absorption spectra of aztreonam and tobramycin are presented in Figure 7. Values of J , E , R_0 and r were determined for every interaction and are reported in Table 5.

Data obtained according to the FRET theory confirm that ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin interact with mucin, whereas the other drugs do not form any complex with the protein. However, the efficiency of energy transfer (E) for the drugs able to form a mucin-drug complex is quite low (0.1-0.3%), while in the other cases E is almost zero. Even if resonance energy transfer (RET) can occur across a distance below 10 nm (Ray et al., 2014), it does not mean that a value of donor-to-acceptor smaller than 10 nm results in RET. Indeed, as for 7-ACA, ampicillin, tobramycin and rifampicin, the donor-to-acceptor value is even more than two-fold the Förster distance (R_0), that is the distance at which the RET is efficient at 50%. Even if rifampicin has a high value of spectra overlap, no interaction is established as the efficiency of energy transfer is zero.

Figure 7. The overlaps of emission spectra of mucin and the absorption spectra of aztreonam (A) and tobramycin (B).

Table 5. Values of J , E , R_0 and r of mucin-drug complexes determined according to equations 3, 4 and 5.

		J (cm ³ L mol ⁻¹)	E (%)	R_0 (nm)	r (nm)
A	7-ACA	$3.82 \cdot 10^{12}$	0.01	3.74	8.56
B	Ceftazidime	$2.99 \cdot 10^{13}$	0.18	5.27	6.75
C	Aztronam	$3.54 \cdot 10^{13}$	0.20	5.41	6.80
D	Ampicillin	$3.36 \cdot 10^{11}$	0.00	2.40	9.07
E	CFTR(inh)-172	$3.24 \cdot 10^{14}$	0.22	7.83	9.69
F	Tobramycin	$3.92 \cdot 10^{10}$	0.00	1.74	4.12
G	Levofloxacin	$1.76 \cdot 10^{14}$	0.13	7.07	9.77
H	Rifampicin	$1.52 \cdot 10^{14}$	0.00	6.90	17.9

4. CONCLUSIONS

In the present work, the interaction of mucin with some antibiotics of interest in cystic fibrosis was investigated at three different temperatures by two spectroscopic methods. Fluorescence quenching data indicate that ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin binds mucin, whereas no interaction is observed for 7-ACA, ampicillin, tobramycin and rifampicin. The increasing quenching upon increasing temperature for ceftazidime and CFTR(inh)-172 indicate a dynamic quenching process. The K_A and n values indicate that ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin moderately binds to mucin and there is only one principal site of binding for this association. The low affinity, or absence of it as for tobramycin, toward mucin could in part explain why these antibiotics are widely used in CF therapies. Contrary to what was expected, the charge of the molecule seems to not play a fundamental role over mucin-drug interaction, in fact positively charged molecules at pH 7.4, such as tobramycin, have no interaction, whereas negatively charged drugs such as CFTR(inh)-172 or aztreonam can complex the protein. Indeed, the thermodynamic parameters (ΔH° and $\Delta S^\circ > 0$) suggest that hydrophobic forces played the major role in the binding process between mucin and ceftazidime, CFTR(inh)-172 and levofloxacin, while electrostatic forces may play a major role for mucin – aztreonam interaction. The negative value of ΔG° implies a spontaneous binding. The distance between donor and acceptor was calculated according to FRET theory and resonance energy transfer was confirmed for ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin. The results herein reported may improve the knowledge about drug pharmacokinetics (absorption and distribution process) for drugs administered in CF therapy. However, in order to find a relation between the molecular structure and affinity towards mucin, further studies should be conducted, and a broader dataset of drugs should be investigated.

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