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RESEARCH ARTICLE

Spectroscopic study on the mechanism of meloxicam and α-amylase

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Abstract: In order to explore the mechanism of action of meloxicam and α-amylase. The interaction between the rheumatoid arthritis drug meloxicam and α-amylase was studied by fluorescence spectroscopy, synchronous fluorescence spectroscopy and molecular docking under the experimental conditions of pH=6.80. The results showed that meloxicam was able to effectively quench the endogenous fluorescence of α-amylase in a static quenching form a 1:1 complex and change the conformation of α-amylase. Thermodynamic results indicated that the main type of meloxicam and α-amylase system was hydrophobic interaction. Molecular docking indicated that the binding system had hydrogen bonds in addition to hydrophobic interaction and meloxicam was surrounded by the active amino acid residues Trp13 and Trp263 of α-amylase, which changed the microenvironment of amino acid residues at the active center of α-amylase. By establishing the binding model, it can be seen that the protein binding rate *W(B)* of meloxicam to -amylase was 2.76%-41.79% under the experimental conditions. The results showed that the binding of meloxicam to -amylase had an effect on the number of free -amylase. The drug binding rate *W(Q)* of the system was 2.76%-1.67%, which indicated that the combination of α-amylase and meloxicam would not affect the efficacy of meloxicam.

Keywords: drug, protein, fluorescence analysis, conformation, molecular docking, binding rate

1 Introduction

Non-steroidal anti-inflammatory drugs can relieve pain and edema to play a role in the treatment of inflammation, however, patients taking non-steroidal anti-inflammatory drugs often cause gastritis, gastric ulcer, kidney and liver damage and other adverse symptoms[1] . Meloxicam (MEL) is one of the non-steroidal anti-inflammatory drugs[2] whose molecular structure is shown in Figure 1. the toxic and side effects of MEL are much less than those of some other non-steroidal anti-inflammatory drugs (such as dotaline, ibuprofen, *etc*.). therefore. The adverse effect of MEL on gastrointestinal function was much smaller[3] . This drug is widely used in daily life and it is a very common drug for the treatment of rheumatoid arthritis. in addition to the treatment of inflammatory diseases, MEL also has a good effect on relieving physical pain in patients[4] .

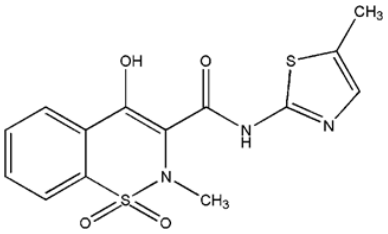

Figure 1. Chemical structure of MEL

α-Amylase (AMS) belongs to a famous group of enzymes, which hydrolyze starch molecules to give diverse products including dextrin and progressively smaller polymers composed of glucose units[5] . They can be found in the body of microorganisms, plants and all of the higher body organisms that use carbohydrates in their metabolism process. Thus, the amylases can be derived from several sources, including plants, animals and microorganisms; microbial enzymes generally meet industrial demands. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry[6] . Over the past few decades, considerable research has been under- taken with the extracellular AMS being produced by a wide variety of microorganisms[7] .

In recent years, fluorescence spectroscopy has become an important means to study the mechanism of ligand-

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protein system. So far, R. Omidyan[8] has studied the interaction between AMS and cetyltrimethylammonium bromide by spectroscopy, and He Qiang[9] has studied the interaction between AMS and tannic acid, a hypoglycemic drug, by spectroscopy. There are many reports on the interaction between small molecular drugs and AMS by spectroscopy. Most of the studies only focus on the binding mechanism of ligands and AMS. However, there are relatively few studies on the binding of AMS to drug molecules by spectroscopy, and then infer the effect of MEL on the efficacy and properties of AMS. In this paper, a series of experimental data were obtained by fluorescence experiments and molecular docking, which revealed the binding mechanism of the system and predicted the drug efficacy and enzyme properties of MEL combined with AMS in human body. The qualitative effect provides a useful reference for the rapid prediction of the interaction between drugs and proteins *in vivo*.

2 Experimental

2.1 Apparatus

RF-5301PC fluorometer (Shimadzu, Japan); UV-3600 UV-vis spectrophotometer (Shimadzu, Japan); SYC-15_Bsuper constant temperature water bath (Nanjing Sanli Electronic equipment Factory); SZ-93 automatic double Pure Water Distiller (Shanghai Yarong biochemical instrument Factory).

2.2 Materials

AMS (purity grade inferior 99%, Sigma), reserve solution (1.0×10^{-5} mol/L); MEL (CAS#,71125-28-7), reserve solution(4.0×10^{-4} mol/L), phosphate buffer solution of pH=6.80 was prepared. The water used in the experiment was secondary quartz distilled water, and the above storage solution was kept away from light at 277 K. The fluorescence signal measured in the experiment was corrected by the "internal filter effect" Equation 1 .[10]

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

Where F_{cor} and F_{obs} are the corrected and observed fluorescence signals, respectively, and A_{ex} and A_{em} are the absorbance values of MEL-AMS system at excitation and emission wavelengths, respectively. The fluorescence signal used in this article was corrected.

2.3 Experiment procedure

2.3.1 Fluorescence experiment

At 298 K, 310 K and 318 K, 1.0 mL phosphate buffer solution, 2.0 mL AMS solution and different volume of MEL solution were added to the 10.0 mL colorimetric tube at constant volume and constant temperature of 30 min. The slit width was 5 nm, λ_{ex} and the scanning fluorescence spectra were 280, 295 nm, respectively. When $\Delta\lambda$=15 nm or 60 nm, scanning synchronous fluorescence spectroscopy.

2.3.2 UV-Vis measurements

At 298 K, 1.0 mL phosphate buffer solution, 2.0 mL AMS solution and different volume MEL solution was added to the 10.0 mL colorimetric tube at constant volume and constant temperature of 30 min. The absorbance of the system was determined by using the corresponding concentration of MEL solution as the blank reference, and the UV absorption spectrum of the system was drawn.

2.3.3 Molecular docking

The crystal structure (PDB ID: 1BLI of AMS comes from the protein database (Protein Data Bank). The ChemDraw Pro 14.0 and ChemBio 3D Ultra 14.0 are used to draw the MEL structure, and the energy minimization of the three-dimensional structure is carried out. AutoDock 4.2.6 was used to study the molecular docking of MEL and AMS, and genetic algorithm was used to calculate the binding conformation of MEL and AMS[11] .

3 Results and discussion

3.1 Fluorescence quenching mechanism studies of MEL-AMS system

The fluorescence effect of protein is produced by the chromophore of Trp, Tyr and Phe residues. The Trp and Tyr residues in protein are excited together at 280 nm wavelength, while at 295 nm wavelength, only Trp residue is excited[12] . Figure 2 showed the fluorescence spectra of the interaction between MEL and AMS (at λ_{ex}=295 nm, the fluorescence spectra of MEL and AMS are similar, but the fluorescence intensity is low). Figure 2 showed that the fluorescence peak of AMS at 343 nm quenched and the emission peak shifts blue with the increase of MEL concentration, indicating that the MEL-AMS system interacted and formed a stable complex.[13]

The Stern-Volmer[14] equation was shown below, through which the quenching constant K_{sv} and the quenching rate constant kq: can be calculated by using the fluorescence signal data obtained from the experiment:

$$F_0/F = 1 + k_q\tau_0[L] = 1 + K_{SV}[L] \quad (2)$$

Where F_0 and F represent the fluorescence signals in the absence and presence of quencher, respectively. τ_0 is the average lifetime of fluorescence without quencher,

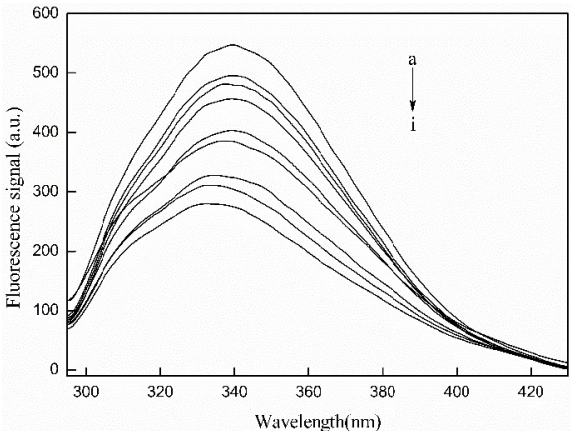

Figure 2. Fluorescence emission spectra of MEL-AMS system (T=310 K, λ_{ex}=280 nm), C_{AMS}=2.0×10^{-6} mol/L, a-i C_{MEL}=(0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0)×10^{-5}mol/L

which is about 10^{-8} s. K_{sv} is the Stern-Volmer quenching constant. k_q is the bimolecular quenching constant, and [L] is the concentration of meloxicam. The results were shown in Table 1. The results showed that the k_q values at different temperatures are larger than the maximum diffusion collision quenching constant of 2 × 10^{10} L/mol·s[15] for biomolecules by various quenching agents. at the same time, it can be seen from the data in Table 1 that with the increase of temperature, k_q of MEL-AMS system and K_{sv}'s. The results showed that the quenching mode of MEL-AMS system is static quenching.

For static quenching, the Equation 3[16] is generally used to calculate the binding constant K_a and the number of binding sites n:

$$\lg\left(\frac{F_0-F}{F}\right)=n\lg K_a+n\lg\left\{[L]-n\frac{F_0-F}{F_0}[B_t]\right\} \quad (3)$$

[B$_t$] represents the concentration of AMS, and the results were shown in Table 1. From Table 1, n ≈ 1 at the experimental temperature indicates that there is only one high affinity binding site[17] for MEL to bind to AMS, that is, MEL forms a 1:1 complex with AMS. The binding constant K_a of MEL to AMS decreased with the increased of temperature, which further proved that the fluorescence quenching type of MEL-AMS system was static quenching. Figure 3 showed the participation of Tyr residues and Trp residues of AMS in MEL-AMS system. The results showed that when λ_{ex}=280 nm and λ_{ex}=295 nm, the quenching curve of MEL-AMS system was separated. This indicated that both Tyr residues and Trp residues in AMS participated in the reaction. However, the slope of the quenching curve of MEL-AMS system at λ_{ex}=295 nm was obviously smaller than that of the binding system at λ_{ex}=280 nm, which indicated that the fluorescence quenching degree of AMS was stronger at λ_{ex}=280 nm.

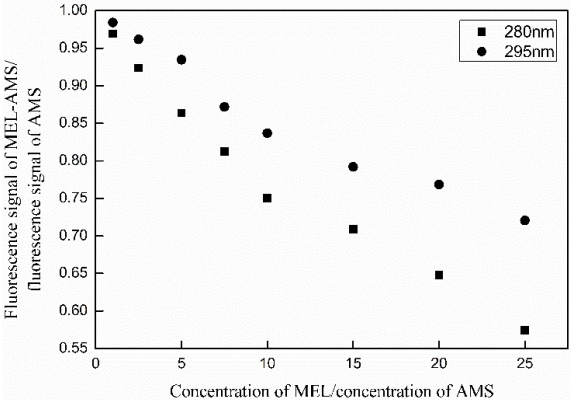

Figure 3. Relative fluorescence curves of the interaction between MEL and AMS (T = 310 K); C_{AMS}=2.0×10^{-6} mol/L, C_{MEL}=(0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0)×10^{-5}mol/L

3.2 Type of interaction force of MEL-AMS system

The thermodynamic parameters of MEL-AMS system are calculated according to van't Hoff equation[18] , and the calculated results were shown in Table 2.

$$R\ln K=\Delta S-\Delta H/T \quad (4)$$

$$\Delta G=-RT\ln K=\Delta H-T\Delta S \quad (5)$$

Where R is a gas constant (ΔH and ΔS with a value of about 8.314), MEL and AMS could be calculated by a linear relationship between the natural logarithm (lnK_a) of the binding constant and the reciprocal (1/T) of the temperature. The results were shown in Table 2. It could be seen from Table 2 that ΔG<0 indicated that the binding reaction between MEL and AMS was spontaneous, and ΔH<0 indicated that the formation of MEL-AMS complex was exothermic. The arrangement of water molecules creates a more random configuration around drugs and proteins in a more orderly manner. Therefore, $\Delta S > 0$ is usually used as evidence of hydrophobic interaction between drug molecules and protein molecules[19] . Based on this, the hydrophobic interaction between MEL and AMS could be judged.

Ross and Subramanian[20] believed that when $\Delta H\approx$ 0, ΔS>0, there was electrostatic attraction between drug molecules and biomolecules, but now some reports thought that when ΔH<0, ΔS>0 could directly judge that the main type of force between the binding systems is electrostatic interaction[21] . In order to further verify whether the main force between MEL and AMS is elec-

Table 1. Quenching reactive parameters of MEL-AMS system at different temperatures

λ_{ex} (nm)	T/(K)	K_{sv} (L/mol·s)	k_q (L/mol)	r_1	K_a (L/mol)	n	r_2
	298	1.70×10^4	1.70×10^{12}	0.9932	1.81×10^4	1.03	0.9922
$\lambda_{ex=}$ 280	310	1.38×10^4	1.38×10^{12}	0.9939	1.46×10^4	0.96	0.9969
	318	1.13×10^4	1.13×10^{12}	0.9957	1.17×10^4	0.98	0.9956
	298	1.36×10^4	1.36×10^{12}	0.9938	1.43×10^4	1.06	0.9942
$\lambda_{ex=}$ 295	310	1.12×10^4	1.12×10^{12}	0.9965	1.08×10^4	0.99	0.9969
	318	0.91×10^4	0.91×10^{12}	0.9974	0.88×10^4	1.02	0.9915

Note: r_1 is the linear relative coefficient of F0/F~[L];

r_2 is the linear relative coefficient of lg[(F0-F)/]~lg{[L]-n[Bt](F0-F)/F0}

trostatic force, the effect of ionic strength on MEL-AMS interaction is discussed in this paper.

Fix the concentration of MEL and AMS, add different concentrations of NaCl, to F/F_0 to map C_{NaCl}, the results were shown in Figure 4. The experimental results showed that when the concentration of NaCl increased, the ratio of F/F_0 did not change significantly. The results showed that the binding of MEL to AMS was not affected by ionic strength, that was, the electrostatic interaction of MEL-AMS system was not obvious. If the electrostatic interaction played a leading role in the binding of protein to ligands, with the concentration of salt in the system. With the increased of the interaction intensity between protein and ligand, the interaction intensity between protein and ligand decreased gradually[22] . This also indicated that when $\Delta H<0$, $\Delta S>0$ could not directly judge the main force between MEL-AMS system was electrostatic force.

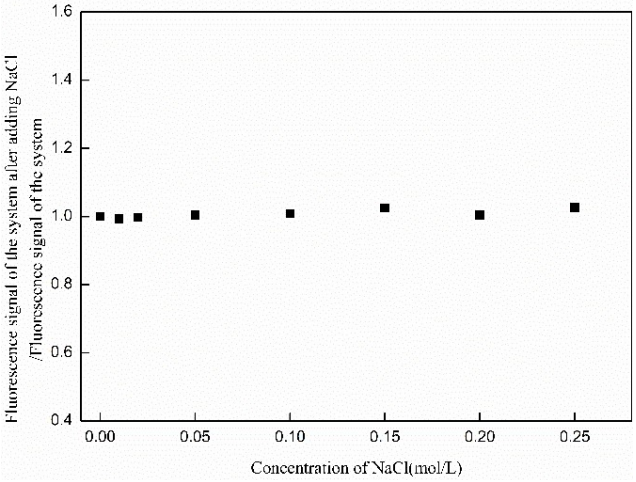

Figure 4. Fluorescence intensity of MEL-AMS system as a function of NaCl concentration (T=298 K); C_{AMS} =2.0×10^{-6} mol/L, C_{MEL}= 2.0×10^{-5}mol/L, C_{Nacl}= (0, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0) ×10^{-1}mol/L

3.3 Conformation studies of MEL-AMS system

3.3.1 Synchronous fluorescence studies of MEL-AMS system

By measuring the shift of the maximum emission wavelength of the synchronous fluorescence spectrum of the binding system, the environmental information of amino acid residues near the fluorescent luminescence group can be explored[23] . As shown in Figure 5, when $\Delta\lambda$=60 nm and $\Delta\lambda$ =15 nm, the intensity of the fluorescence signal of the AMS tended to decrease, and the fluorescence peaks of both the Trp residue and the Tyr residue shifted very slightly, this means that the binding of MEL to AMS changes the microenvironment of amino acid residues in small molecular proteins such as AMS, reducing its hydrophobicity. The extension of peptide chain increased[24] and Tyr and Trp residues were involved in the binding reaction. This result was consistent with conclusion 3.1.

Table 2. The thermodynamic parameters of MEL-AMS at different temperatures

System	T/(K)	K_a /(L/mol)	ΔH/(kJ/mol)	ΔS/(J/mol·K)	ΔG/(kJ/mol)
$\lambda_{ex=}$	298	1.81×10^4		24.49	-24.29
	310	1.46×10^4		24.74	-24.66
280 nm	318	1.17×10^4	-16.99	24.45	-24.77

3.3.2 UV-vis absorption spectra studies of MEL-AMS system

UV-vis absorption spectra can be used to explore the structural changes of proteins and to study the formation of protein-ligand complexes[25] . Figure 6 was an ab-

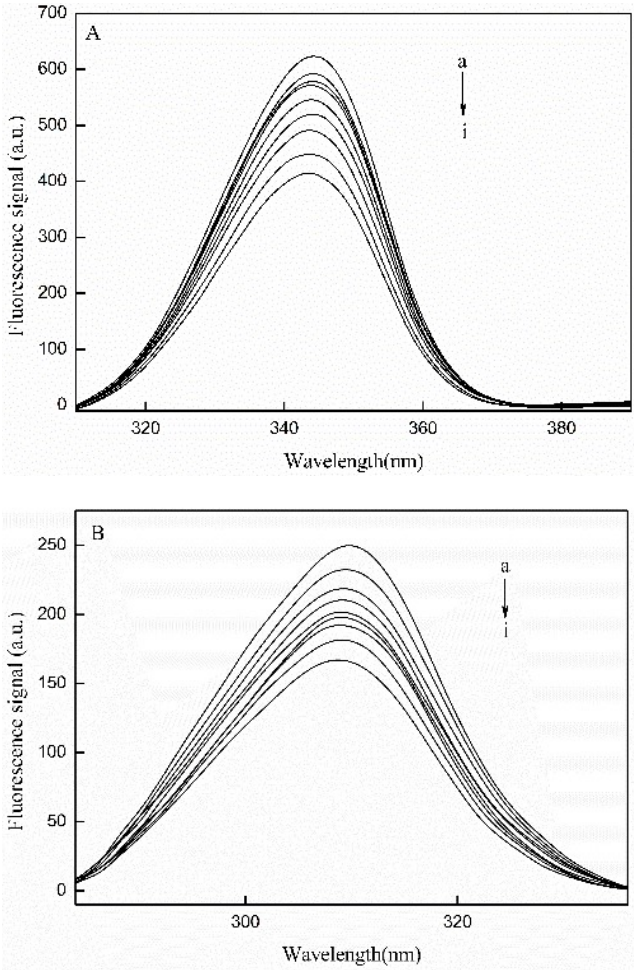

Figure 5. Synchronous fluorescence spectra of MEL-AMS system (T=310 K). (A) $\Delta\lambda$=60 nm; (B) $\Delta\lambda$=15nm; C_{AMS}=2.0×10^{-6} mol/L, a-i C_{MEL}=(0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0)×10^{-5}mol/L

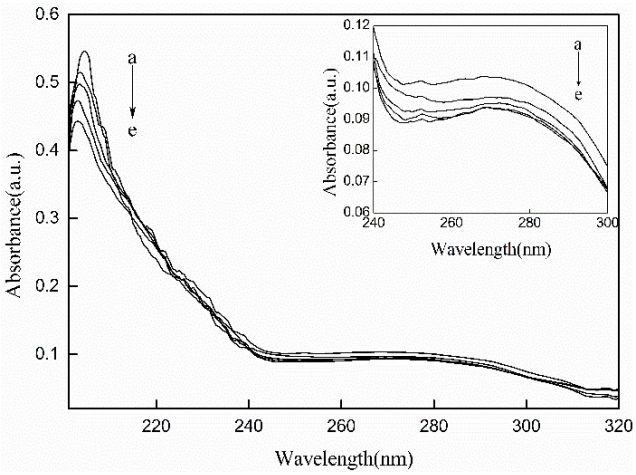

Figure 6. Fluorescence intensity of MEL-AMS system as a function of NaCl concentration (Absorption spectra of MEL-AMS system (T=298 K); C_{AMS}=2.0×10^{-6} mol/L, a-e C_{MEL}= (0, 0.2, 0.5, 1.0, 2.0)×10^{-5}mol/L

sorption spectrum of the MEL-AMS system. from the figure, it could be seen that AMS has two absorption peaks, and its strong absorption peak near 208 nm reflected the frame conformation of the protein. The weak absorption peak at about 280 nm was due to aromatic amino acids (Trp, Tyr and Phe)[26] . With the increased of MEL concentration, Figure 6 showed that the intensity of the absorption peak at 208nm decreased with the blue shifted, and the absorption peak at 280nm also decreased slightly. This result indicated that the interaction between MEL and AMS led to the formation of new complexes, and the AMS molecule tends to fold, the hydrophobicity of AMS microenvironment was enhanced.

3.4 Molecular docking

Molecular docking plays an important role in exploring the interaction between ligands and receptors. In order to further determine the binding position of MEL-AMS system and the effect of MEL binding to AMS on ligands and receptors. In this paper, the binding model of MEL and AMS was established by molecular docking method. By this method, the type of force and the lowest binding energy of MEL and AMS binding system could be obtained. Figure 7(A) showed the optimal binding position after MEL binds to AMS, the Asp328 residue forms two hydrogen bonds with MEL with bond lengths of 2.138 $\mathring{A}$ and 2.144 $\mathring{A}$ respectively, and the His253 residue formed a hydrogen bond with MEL. The bond length is 1.791 $\mathring{A}$, which showed that the hydrogen bond played an important role in the binding of MEL to AMS. Figure 7(B) showed a plurality of hydrophobic amino acid residues such as Ala232, Tyr262, Trp13, Trp263, Leu196, Leu335 and Val233 around MEL, further indicated that there was hydrophobic force in the binding process between MEL and AMS. The amino acid residues such as Tyr262, Trp13 and Trp263 were relatively close to the binding position of MEL and AMS, which led to the binding could effectively quench the endogenous fluorescence of AMS, which was consistent with the conclusion of fluorescence quenching experiment. Trp13 and Trp263 were the key residues of the catalytic active center of AMS[27] . The results of molecular docking also showed that the binding of MEL and AMS could change the microenvironment of the catalytic active center of AMS. In other words, the binding of the system might affect the catalytic activity of AMS.

The binding energy obtained from molecular docking for MEL and AMS interaction was -25.89 kJ/mol. Whereas, the free energy change calculated from fluorescence quenching results was -24.66 kJ/mol at 310 K.

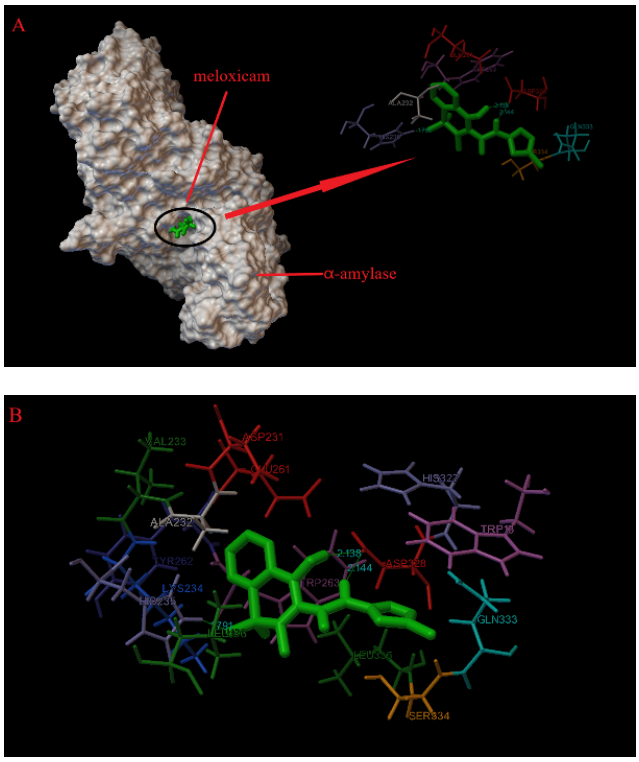

Figure 7. Theoretical Modeling of the Interaction between MEL and AMS; (A) MEL located within the hydrophobic pocket in AMS; (B) Detailed illustration of the amino acid residues lining the binding site in the MEL and AMS cavity

This difference may be due to exclusion of the solvent in docking simulations or rigidity of the receptor other than Trp and Tyr residues[28] . The energy data obtained by docking the molecules were listed in Table 3. From Table 3, it could be also seen that the electrostatic energy was very much lower than the sum of van der Waals energy, hydrogen bonding energy and desolvation free energy in the binding process of MEL with AMS, indicating that the main interaction mode between MEL and AMS was not electrostatic binding mode.Combined with the data of fluorescence experiments and the results of theoretical modeling, it could be seen that hydrophobic interaction and hydrogen bond were the main forces driving the combination of MEL molecules with AMS molecules, which led to the static quenching of AMS.

3.5 MEL-AMS system binding rate

The study of protein binding rate and drug binding rate of drug-protein system is helpful to further study the interaction between drug molecules and protein molecules, so that drug molecules can bind to some proteins in human body. After action, a simple prediction can be made of the efficacy of the drug, the properties and effects of the protein itself, and the effects on the physiological function of the human body. According to the fluorescence experiment of K_a, the drug binding rate and protein binding rate of MEL to AMS can be calculated. When n=1, the binding rate (W) formula of MEL-AMS system was as follows[29] .

Table 3. Docking energy of MEL-AMS system (unit: kJ/mol)

Protein PDB ID	ΔG_0	ΔE_1	ΔE_2	ΔE_3
1BLI	-25.89	-29.65	-28.81	-0.84

ΔG_0 is the binding energy in the binding process.
ΔE_1 denotes intermolecular interaction energy, which is a sum of van der Waals energy, hydrogen bonding energy, desolvation free energy and electrostatic energy.
ΔE_2 is the sum of van der Waals energy, hydrogen bonding energy and desolvation free energy.
ΔE_3 is the electrostatic energy.

Drug binding rate:

$$W(Q) = \frac{x}{Q} \times 100\% = \frac{K_a(Q+B)+1-\sqrt{K_a^2(Q-B)^2+2K_a(Q+B)+1}}{2K_aQ} \times 100\% \quad (6)$$

Protein binding rate:

$$W(B) = \frac{x}{B} \times 100\% = \frac{K_a(Q+B)+1-\sqrt{K_a^2(Q-B)^2+2K_a(Q+B)+1}}{2K_aB} \times 100\% \quad (7)$$

Wherein Q represents the total concentration of MEL and B represents the total concentration of AMS. At the three temperatures of 298K, 310K and 318K, the drug binding rate $W(Q)$ of MEL and AMS calculated according to formulas (6) and (7) was 3.38%-1.88% (298K), 2.76%-1.67% (310K), 2.24%-1.46% (318K), protein binding rate $W(B)$ was 3.38%-47.03% (298K), 2.76%-41.79% (310K), 2.24%~36.57% (318K). At three temperatures, the drug binding rate and protein binding rate of MEL to AMS decreased with the increase of temperature, and the content of free drugs and proteins increased with the increase of temperature, which indicated that the stability of the binding system decreased with the increase of temperature. The results were consistent with the experimental results of fluorescence quenching.

The ratios of $W(Q)$ and $W(B)$ to drug concentration and protein concentration were plotted, respectively, as shown in Figure 8. It could be seen from Figure 8 that the $W(Q)$ value decreased with the increased of temperature, while the $W(B)$ value was large and increased. Taking 310K data close to human body temperature as an example: The $W(Q)$ of MEL and AMS was 2.76%-1.67%, which indicated that the interaction between MEL and AMS had little effect on the efficacy of MEL as the

amount of MEL added increases. The $W(B)$ of MEL and AMS was 2.76%-41.79%, which indicated that the protein binding rate varies greatly with the increase of MEL addition. Combined with the results of 3.4, MEL might have an effect on the activity of AMS, which was not conducive to the digestion and absorption of starchy substances. Therefore, the intake of drugs should be strictly controlled when taking MEL, so as to reduce the adverse effects of drugs on the digestive system.

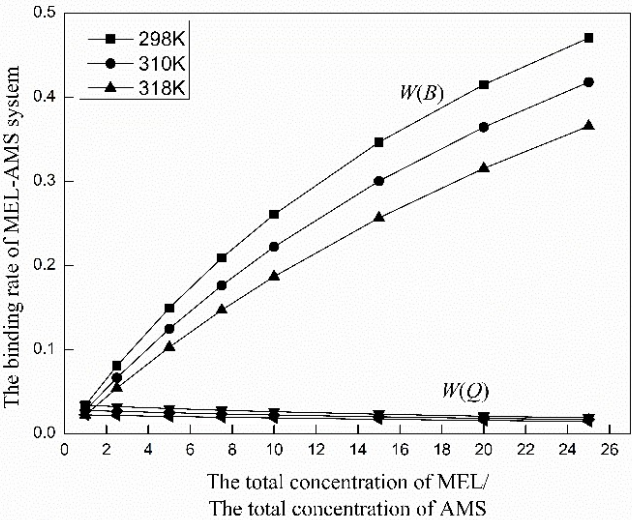

Figure 8. The binding rate of MEL to protein in different temperature and the binding rate of AMS to drug (λ_{ex}=280 nm)

4 Conclusion

In this paper, the interaction between MEL and AMS was studied by spectroscopic and theoretical modeling under simulated physiological conditions. The binding rate model of MEL and AMS was established. the interaction between MEL and AMS was used to treat drugs. The effects of AMS on the digestive function of starches were simply predicted. It provides a new idea to study the effect of the interaction between protein molecules and small drug molecules on the properties of proteins and the efficacy of drugs. The binding rate of ligand-protein system is studied by spectroscopy. Compared with other methods such as equilibrium dialysis, there will be some errors in the binding constant and the number of binding sites, but these commonly used binding rates are studied. The experimental equipment of the method is expensive, the experimental period is long and the concentration range of the experimental drug is narrow. However, the method of studying the binding rate by spectroscopy is simple, rapid and suitable for a wide range of applications, so this method is preferable.

References

[1] Franze JA, Carvalho TF, Galieri C, *et al*. Synthesis, characterization, thermal and spectroscopic studies and bioactivity of complexes of meloxicam with some bivalent transition metals. Journal of Thermal Analysis & Calorimetry, 2017, **127**(2): 1393-1405. http://doi.org/10.1007/s10973-016-6030-5

[2] Sayen S, Carlier A, Tarpin M, *et al*. A novel copper (II) mononuclear complex with the non-steroidal anti-inflammatory drug diclofenac: structural characterization and biological activity. Journal of Inorganic Biochemistry, 2013, **120**(3): 39-43. http://doi.org/10.1016/j.jinorgbio.2012.12.002

[3] Shantiaee Y, Javaheri S, Movahhedian A, *et al*. Efficacy of preoperative ibuprofen and meloxicam on the success rate of inferior alveolar nerve block for teeth with irreversible pulpitis. International Dental Journal, 2017, **67**(2): 85-90. http://doi.org/10.1111/idj.12272

[4] Ebrahimi M, Khayamian T, Hadadzadeh H, *et al*. Spectroscopic, biological, and molecular modeling studies on the interactions of [Fe (III)-meloxicam] with G-quadruplex DNA and investigation of its release from bovine serum albumin (BSA) nanoparticles. Journal of Biomolecular Structure and Dynamics, 2015, **33**(11): 2316-2329. http://doi.org/10.1080/07391102.2014.1003195

[5] Park KH, Kim TJ, Cheong TK, *et al*. Structure, specificity and function of cyclomaltodextrinase, a multispecific enzyme of the α-amylase family. Biochimica Et Biophysica Acta, 2000, **1478**(2): 165-185. http://doi.org/10.1016/S0167-4838(00)00041-8

[6] Pandey A, Nigam P, Soccol CR, *et al*. Advances in Microbial Amylases. Biotechnology and Applied Biochemistry, 2000, **31**(2): 135-152. http://doi.org/10.1042/BA19990073

[7] Gupta R, Gigras P, Mohapatra H, *et al*. Microbial α-amylases: a biotechnological perspective. Process Biochemistry, 2003, **38**(11): 1599-1616. http://doi.org/10.1016/S0032-9592(03)00053-0

[8] Omidyan R, Kazemi SH, Bordbar AK, *et al*. Spectroscopic study on the interaction of Bacillus subtilis α-amylase with cetyltrimethylammonium bromide. Journal of Luminescence, 2011, **131**(6): 1229-1233. http://doi.org/10.1016/j.jlumin.2011.02.001

[9] Lin FY and Qiang HE. Interaction between tannic acid and pancreatic α-amylase. Science & Technology of Food Industry, 2014, **35**(3): 63-66. http://doi.org/10.13386/j.issn1002-0306.2014.03.040

[10] Rakotoarivelo NV, Perio P, Najahi E, *et al*. Interaction between Antimalarial 2-Aryl-3H-indol-3-one Derivatives and Human Serum Albumin. The Journal of Physical Chemistry B, 2014, **118**: 13477-13485. http://doi.org/10.1021/jp507569e

[11] Elmas G and Esra Y. Fluorescence interaction and determination of sulfathiazole with trypsin. Journal of Fluorescence, 2014, **24**(5): 1439-1445. http://doi.org/10.1007/s10895-014-1427-7

[12] Zhang LH, Liu BS, Li ZY, *et al*. Comparative studies on the interaction of cefixime with bovine serum albumin by

fluorescence quenching spectroscopy and synchronous fluorescence spectroscopy. Asian Journal of Chemistry, 2015, **30**(5): 686-692.
http://doi.org/10.1002/bio.2805

[13] Mahaki H, Memarpoor-Yazdi M, Chamani J, *et al.* Interaction between ropinirole hydrochloride and aspirin with human serum albumin as binary and ternary systems by multi-spectroscopic, molecular modeling and zeta potential. Journal of Luminescence, 2013, **134**(3): 758-771.
http://doi.org/10.1016/j.jlumin.2012.06.051

[14] Safarnejad A, Shaghaghi M, Dehghan G, *et al.* Binding of carvedilol to serum albumins investigated by multi-spectroscopic and molecular modeling methods. Journal of Luminescence, 2016, **176**: 149-158.
http://doi.org/10.1016/j.jlumin.2016.02.001

[15] Moeiopour F, Mohseni-Shari FS, Malaekeh-Nikouei B, *et al*. Investigation into the interaction of losartan with human serum albumin and glycated human serum albumin by spectroscopic and molecular dynamics simulation techniques: acomparison study. Chemico-Biological Interactions, 2016, **257**: 4-13.
http://doi.org/10.1016/j.cbi.2016.07.025

[16] Cao SN, Liu BS, Li ZY, *et al.* A fluorescence spectroscopic study of the interaction between glipizide and bovine serum albumin and its analytical application. Journal of Luminescence,2014, **145**(31): 94-99.
http://doi.org/10.1016/j.jlumin.2013.07.026

[17] Amroabadi MK, Taheri-Kafrani A, Saremi LH, *et al*. Spectroscopic Studies of the interaction between alprazolam and apo-human serum transferrin as a drug carrier protein. International Journal of Biological Macromolecules, 2017, **108**: 263-271.
http://doi.org/10.1016/j.ijbiomac.2017.11.179

[18] Hu Y, Yang Y, Dai C, *et al*. Site-Selective Binding of human serum albumin by palmatine: spectroscopic approach. Biomacromolecules, 2010, **11**(1): 106-112.
http://doi.org/10.1021/bm900961e

[19] Abdus-Salam M, Rokonujjaman M, Rahman A, *et al.* Study of in Vitro Interaction of Sildenafil Citrate with Bovine Serum Albumin by Fluorescence Spectroscopy. Pharmacology & Pharmacy, 2015, **6**(2): 94-101.
http://doi.org/10.4236/pp.2015.62012

[20] Ross PD and Subramanian S. Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry, 1981, **20**(11): 3096-3102.
http://doi.org/10.1021/bi00514a017

[21] Ma X, Song B, Xiao W, *et al*. Study on Interaction between Alliin and Bovine and Hunman Serum Albumin with Spectrometry. Spectroscopy and Spectral Analysis, 2017, **37**(6): 1826-1830.
http://doi.org/j.issn.1000-0593(2017)06-1826-05

[22] Zhou HF, Bi SY, Wang Y, *et al*. Characterization of the binding of paylean and DNA by fluorescence, UV spectroscopy and molecular docking techniques. Luminescence, 2016, **1**(4): 1013-1019.
http://doi.org/10.1002/bio.3066

[23] Buddanavar AT and Nandibewoor ST. Multi-spectroscopic characterization of bovine serum albumin upon interaction with atomoxetine. Journal of Pharmaceutical Analysis, 2017, **7**(3): 148-155.
http://dx.doi.org/10.1016/j.jpha.2016.10.001

[24] Xu CB, Gu JL, Ma XP, *et al*. Investigation on the interaction of pyrene with bovine serum albumin using spectroscopic methods. Pectrochimica Acta Part A-Molecular and Biomolecular Spectroscopy, 2014, **125**(9): 391-395.
http://doi.org/10.1016/j.saa.2014.01.132

[25] Chi ZX and Liu RT. Phenotypic Characterization of the Binding of Tetracycline to Human Serum Albumin. Biomacromolecules, 2011, **12**(1): 203-209.
http://doi.org/10.1021/bm1011568

[26] Hu XX, Yu ZH and Liu RT. Spectroscopic investigations on the interactions between isopropanol and trypsin at molecular level. Spectrochim Acta A Mol Biomol Spectrosc, 2013, **108**: 50-54.
http://doi.org/10.1016/j.saa.2013.01.072

[27] Najafian M, Ebrahim-Habibi A, Hezareh N, *et al*. Transchalcone: A novel small molecule inhibitor of mammalian alpha-amylase. Molecular Biology Reports, 2011, **38**(3): 1617-1620.
http://doi.org/10.1007/s11033-010-0271-3

[28] Jana S, Dalapati S, Ghosh S, *et al*. Study of microheterogeneous environment of protein human serum albumin by an extrinsic fluorescent reporter: a spectroscopic study in combination with molecular docking and molecular dynamics simulation. Journal of Photochemistry & Photobiology B Biology, 2012, **112**(231): 48-58.
http://doi.org/10.1016/j.jphotobiol.2012.04.007

[29] Ma LH, Liu BS, Wang CD, *et al*. The interaction mechanism of nifedipine and pepsin. Monatshefte für Chemie-Chemical Monthly, 2018, **149**(11): 2123-2130.
http://doi.org/10.1007/s00706-018-2269-9

