

姜黄素和去甲氧基姜黄素与牛血清白蛋白相互作用热力学行为

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2016-04-12 收稿, 2016-05-16 修回, 2016-05-18 接受, 2016-08-12 网络版发表

湖北省卫生和计划生育委员会重点项目(WJ2015MB097)和武汉黄鹤英才(科技)计划(2014[10])资助

摘要 在模拟人生理条件下, 综合利用荧光光谱、圆二色谱和分子模拟等方法, 研究姜黄素及其衍生物去甲氧基姜黄素与牛血清白蛋白(BSA)相互作用的热力学特征. 荧光光谱分析表明, 姜黄素和去甲氧基姜黄素均能有效猝灭BSA的内源荧光, 猝灭机制属于静态猝灭. 通过计算, 获取了相应的热力学参数, 证明2种药物与BSA的相互作用是一个吉布斯自由能降低的自发过程, 且二者之间的主要作用力为疏水作用力. 位点竞争实验和分子模拟的结果表明, 2种药物在BSA的主要结合位点均为Site I. 圆二色谱的分析发现, 2种药物使BSA的构象发生了改变.

关键词 姜黄素, 去甲氧基姜黄素, 牛血清白蛋白, 热力学参数, 荧光光谱, 圆二色谱, 分子对接

姜黄素(curcumin)是一种从姜科植物姜黄的根茎中提取得到的酚类色素, 是姜黄发挥药理作用最重要的活性成分^[1~3]. 研究证实姜黄素及其衍生物具有广泛的药理作用, 如抗炎、抗氧化、降血脂、抗动脉粥样硬化、抗肿瘤、抗HIV病毒等^[4~6]. 姜黄素还可以保护脂质、血红蛋白和DNA等不被氧化降解^[7]. 姜黄素无毒副作用, 并具有广谱的生物活性, 但是它在中性溶液中溶解性较差, 从而限制了其生物利用度和临床效率. 姜黄素与许多生物大分子(如载体蛋白)结合, 可以提高溶解性和稳定性, 并在结合状态下仍保留其生物活性^[8,9].

血清白蛋白是血浆中含量最多的载体蛋白, 具有维持人体生理环境相对稳定、贮运外源性药物小分子和内源性代谢物等重要生理功能^[10~12]. 药物分子进入机体血液后, 首先与血清白蛋白进行不同程度

地结合, 通过血清白蛋白进行储存及运输, 可控地向受体运输和释放, 进而发生药理作用^[13]. 因此, 研究药物分子与血清白蛋白之间的相互作用, 将有益于了解药物分子在机体内的储存、运输及释放机制, 为药理学信息和药物研发提供理论性的启发与指导^[14].

研究报道姜黄素在生理缓冲溶液条件下会迅速地水解从而丧失生理活性, 但是在人体内, 姜黄素与血清蛋白结合会提高其稳定性, 明显地抑制其水解过程^[15]. Leung和Kee^[16]采用光谱法研究了姜黄素与血清白蛋白(HSA)、纤维蛋白原、转铁蛋白等几种常见的血清蛋白质的结合能力, 发现姜黄素与血清白蛋白和纤维蛋白原的结合常数比其他血清蛋白质的结合常数大1~2个数量级, 而且与这2种蛋白质结合之后水解速率降低95%以上. Mandeville等人^[17]通过傅里叶变换红外和圆二色谱的手段发现姜黄素会在

引用格式: 郭清莲, 潘凌立, 杨立云, 等. 姜黄素和去甲氧基姜黄素与牛血清白蛋白相互作用热力学行为. 科学通报, 2016, 61: 3137~3145

Guo Q L, Pan L L, Yang L Y, et al. Thermodynamics of the interaction of curcumin and demethoxycurcumin with bovine serum albumin (in Chinese). Chin Sci Bull, 2016, 61: 3137~3145, doi: 10.1360/N972016-00302

一定程度上诱导HSA去折叠. Barik等人^[18]的研究表明姜黄素与HSA 214位色氨酸会发生荧光共振能量转移(FRET), 估算二者的距离为2.74 nm. 这些研究为了解姜黄素在人体内的吸收代谢行为提供了重要的信息, 但目前对姜黄素衍生物与血清蛋白结合行为的研究报道较少, 其构效关系的研究非常稀缺. 研究姜黄素及其衍生物与血清蛋白的相互作用机制并进行比较, 对于了解姜黄素在体内的作用机制有重要意义, 对于姜黄素分子结构的改造和设计也有重要的帮助.

由于牛血清白蛋白(BSA)与人血清白蛋白具有高度的同源性, 本文以牛血清白蛋白为模型, 利用荧光、紫外、圆二色等多种光谱手段和分子模拟的方法, 研究了血清白蛋白与姜黄素(curcumin, 图1)及去甲氧基姜黄素(demethoxycurcumin, 图1)相互作用的热力学特征及其机理.

1 实验

(i) 试剂与仪器. 牛血清白蛋白(bovine serum albumin, BSA), 布洛芬(Ibuprofen)为Sigma公司产品; 华法林(Warfarin)为江苏医药有限公司产品; HCl, NaCl, NaH₂PO₄, K₂HPO₄均购自国药集团, 纯度为分析纯; 姜黄素(HPLC>98%)、去甲氧基姜黄素(demethoxycurcumin, HPLC>98%)均为上海顺勃生物工程公司产品; 实验用水为超纯水, 由Milli-Q Advantage A10超纯水系统获取; BSA等溶液均用浓度为0.05 mol/L, pH 7.4的磷酸盐缓冲溶液(PBS)配成.

荧光光谱由附带恒温水浴的荧光光度计(美国Perkin Elmer公司, LS-55型)测定, 蛋白质圆二色由英国应用光物理公司圆二色谱光谱仪测定. 实验当中固体化学物质由电子分析天平称取(北京赛多利斯仪器系统有限公司, BS110S型).

计算模拟采用Tripos公司Sybyl 8.1软件中的

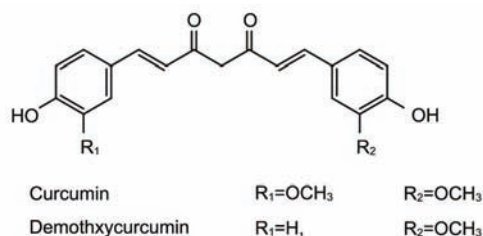


图1 姜黄素及去甲氧基姜黄素的分子结构图

Figure 1 Molecular structure of Curcumin and Demethoxycurcumin

Surflex-Dock模块, 运行系统环境为Cent OS.

(ii) 荧光光谱. 在pH 7.4条件下, 选定荧光激发波长为 $\lambda_{\text{ex}}=280 \text{ nm}$ ^[19], 测定BSA与一系列不同浓度的姜黄素及其衍生物的荧光发射光谱^[20], 波长范围为300~480 nm.

位点竞争实验: 采用荧光滴定法, 固定BSA和位点竞争剂(Warfarin, Ibuprofen)的浓度为 $1.0 \times 10^{-5} \text{ mol L}^{-1}$, 分别将姜黄素和去甲氧基姜黄素逐渐加入BSA-Warfarin或BSA-Ibuprofen混合溶液中, 参数与荧光猝灭实验相同, 分别测定2个体系在波长范围为300~480 nm内的荧光发射光谱.

(iii) 圆二色谱. 室温时, 持续氮气流条件下, 测定波长范围为200~260 nm内样品的圆二色谱, 比色皿的光路长为0.1 cm, 扫描速度200 nm/min. 在相同实验条件下, 测定空白缓冲溶液的圆二色谱, 作为参比扣除. 蛋白质 α -螺旋结构的相对含量根据208 nm处的平均摩尔椭圆率(MRE, 单位为degree cm² mol⁻¹)值依下式计算^[21]:

$$\alpha\text{-螺旋结构的含量} = \frac{-\text{MRE}_{208} - 4000}{33000 - 4000} \times 100\%, \quad (1)$$

式中, MRE₂₀₈为实验条件下测定的208 nm处MRE值; 4000为 β -折叠、无规卷曲构象在208 nm处的MRE值; 33000为单纯的 α -螺旋结构在208 nm处的MRE值.

(iv) 分子对接. 本实验中BSA晶体结构来自数据库Protein Data Bank(PDB), 编号为3v03, 原始晶体结构数据经去水、加氢、加电荷等处理. 小分子采用最小能量法简单优化, 三维结构由软件Sybyl 8.1构建.

2 结果与讨论

2.1 荧光猝灭机制及猝灭常数

荧光猝灭是指分子间相互作用, 如能量转移、激发态反应、形成基态复合物、分子重排及碰撞等, 导致荧光物质荧光强度降低的现象^[22]. 荧光猝灭过程通常可以分为静态猝灭和动态猝灭, 通过温度对结合常数和黏度的影响及测定荧光寿命的方法可以区分不同的猝灭机制^[23]. 动态猝灭主要依赖于分子扩散, 结合常数会随着温度的升高而增大; 然而对于静态猝灭, 温度升高会降低基态配合物的稳定性, 结合常数减小^[24].

图2给出了pH 7.4时不同浓度的姜黄素及其衍生

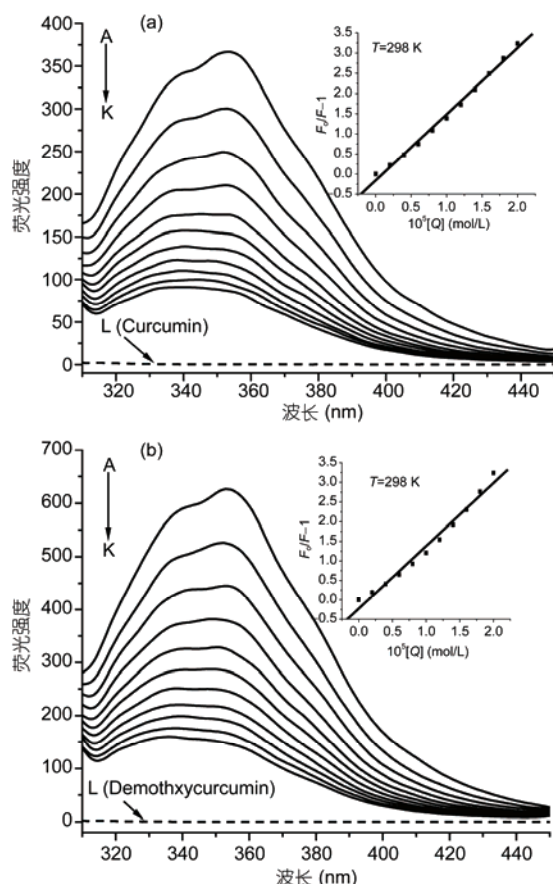


图2 姜黄素(a)及其衍生物去甲氧基姜黄素(b)对牛血清白蛋白荧光光谱的影响及其Stern-Volmer关系图($T=298\text{ K}$, $\lambda_{\text{ex}}=295\text{ nm}$). $c(\text{BSA})=1.0\times 10^{-5}\text{ mol/L}$; 从A到K, $c(\text{curcumin})=c(\text{demethoxycurcumin})=0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0\times 10^{-5}\text{ mol/L}$; 线L为 10^{-5} mol/L 姜黄素或去甲氧基姜黄素的荧光发射光谱

Figure 2 Effects of curcumin (a) and its derivative Demethoxycurcumin (b) on fluorescence spectra of BSA and corresponding Stern-Volmer plots. $c(\text{BSA})=1.0\times 10^{-5}\text{ mol/L}$; from A to K, $c(\text{curcumin})=c(\text{demethoxycurcumin})=0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0\times 10^{-5}\text{ mol/L}$; curve L shows the emission spectrum of 10^{-5} mol/L curcumin or demethoxycurcumin only

物与BSA相互作用的荧光光谱。从图2可以看出, 当 $\lambda_{\text{ex}}=280\text{ nm}$ 时, BSA在 350 nm 附近有1个强的荧光发射峰, 而这2种药物在 350 nm 附近没有荧光发射(线L), 表明这2种药物不会对BSA的内源荧光产生干扰。随着药物的不断加入, BSA的荧光强度逐渐降低, 其内源荧光产生了不同程度的猝灭。

为了判断姜黄素及其衍生物与BSA相互作用体系的猝灭机制, 采用经典的Stern-Volmer方程, 分析了3个不同温度条件下($298, 304, 310\text{ K}$)的荧光猝灭数据^[25]:

$$F_0/F = 1 + K_{\text{sv}}[Q] = 1 + k_q\tau_0[Q], \quad (2)$$

其中, K_{sv} 为Stern-Volmer猝灭常数, k_q 为生物大分子的

猝灭速率常数, $[Q]$ 为猝灭剂的浓度, τ_0 为荧光分子平均寿命, 生物大分子的平均寿命为 10^{-8} s ^[14]。应用式(1)对BSA的荧光数据和姜黄素及其衍生物的浓度进行线性拟合, 由此计算不同温度下的猝灭常数, 结果列于表1。

从表1中的数据可以看出, 两种药物的猝灭常数均随着温度的升高而减小, 说明两种药物对BSA的荧光猝灭均属静态猝灭机理。

采用修正的Stern-Volmer方程, 进一步分析静态荧光猝灭数据^[26]:

$$F_0/\Delta F = \frac{1}{f_a K_a [Q]} + \frac{1}{f_a}, \quad (3)$$

式中, f_a 为荧光团可接近猝灭剂的比例, ΔF 为无猝灭剂与猝灭剂浓度为 $[Q]$ 时体系的荧光强度差值, K_a 为有效猝灭常数, 即结合常数。表2列出了不同温度下的 K_a 值, 其变化趋势与 K_{sv} 的变化趋势一致, 表明两种药物小分子确实与BSA结合生成了复合物。其结合常数较大, 说明姜黄素和去甲氧基姜黄素与血清白蛋白有强的结合, 有利于药物通过血液循环到达作用部位, 从而发挥药效作用。在两种药物中, 姜黄素与BSA的结合常数较大, 结合较强; 去甲氧基姜黄素由于结构中甲氧基的去除, 与BSA作用的结合常数减小, 结合作用减弱。

2.2 相互作用力类型

药物小分子与生物大分子之间相互作用力, 通常包括氢键、范德华力、静电作用力、疏水作用力等^[27]。假如相互作用的焓变(ΔH)在所研究的温度范围内为常数或变化不大, 其值可由范特霍夫方程求出:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}, \quad (4)$$

吉布斯自由能变(ΔG)由下式计算:

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

表1 不同温度姜黄素及其衍生物去甲氧基姜黄素与BSA相互作用的猝灭常数

Table 1 Quenching constants of the interaction between curcumin and demethoxycurcumin with BSA at different temperatures^{a)}

$T(\text{K})$	Curcumin-BSA		Demethoxycurcumin-BSA	
	$K_{\text{sv}}(10^{-5}, \text{L/mol})$	R	$K_{\text{sv}}(10^{-5}, \text{L/mol})$	R
298	1.52	0.999	1.61	0.998
304	1.28	0.999	1.57	0.998
310	1.06	0.999	1.41	0.997

a) R , 相关系数

式中, K 与相应温度下的有效猝灭常数 K_a 相似, R 为气体常数. 如图3所示, $\ln K$ 与 $1/T$ 呈良好的线性关系. 由式(4)和(5)得到焓变和熵变值, 列于表2. 姜黄素和去甲基姜黄素与BSA在298 K条件下相互作用 ΔG 分别为-28.13和-27.42 kJ/mol, 表明他们与BSA在溶液当中的结合过程容易进行, 而且甲氧基的移除降低的姜黄素与BSA结合常数. 此外, ΔH 和 ΔS 均大于0, 根据Ross理论^[28]可以推断姜黄素和去甲氧基姜黄素与BSA之间的作用力主要是疏水作用力.

2.3 作用位点

BSA是一个心形螺旋结构, 其结构可分为3个区域(I, II和III), 每个区域又有2个亚域(A和B), 在亚域II A和亚域III A的疏水腔内, 有2个典型的结合位点Site I和Site II^[29]. 通常选择2种常见的典型位点标记物华法林(Warfarin)和布洛芬(Ibuprofen)分别作为Site I和Site II位点的标记物加入到BSA体系中, 再研究姜黄素或去甲氧基姜黄素与BSA的结合作用, 来确定2种药物小分子在BSA上的结合位置.

如图4所示, 当向BSA溶液中加入华法林时, BSA的荧光最大发射峰波长发生了明显的红移, 荧光强度也发生了增敏; 继续向上述BSA和华法林的混合溶液(摩尔比为1:1)中滴加姜黄素或去甲氧基姜黄素后, 荧光强度显著下降; 随着体系中药物浓度的增加, 其荧光产生有规律的猝灭. 而布洛芬加入BSA的溶液中之后, BSA的荧光强度并没有显著的改变, 随着药物浓度的加入, 其荧光也产生有规律的猝灭.

为了便于比较, 采用修正的Stern-Volmer方程, 对实验数据进行了处理, 结果列于表3. 可以看出, 华法林存在时的结合常数远远大于只有BSA存在时的体系的结合常数. 而用布洛芬标记物进行实验的体系中, 姜黄素和去甲氧基姜黄素与BSA相互作用的结合常数无较大差异. 由实验结果推断两种药物在BSA上的结合位置均为site I.

2.4 姜黄素及其衍生物对BSA构象的影响

药物分子与蛋白质的相互作用可能会引起蛋白质构象的变化, 而圆二色谱是分析蛋白质构象

表2 姜黄素及其衍生物去甲氧基姜黄素与BSA作用体系的相关热力学参数

Table 2 Relative thermodynamic parameters of curcumin-BSA and demethoxycurcumin-BSA systems

体系	T(K)	$K_a(\times 10^4 \text{ L/mol})$	$\Delta H(\text{kJ/mol})$	$\Delta G(\text{kJ/mol})$	$\Delta S(\text{J/(mol K)})$	R	SD ^{a)}
Curcumin-BSA	298	8.53	21.21	-28.13	163.58	0.999	0.0323
	304	10.18		-29.14		0.998	0.0324
	310	11.88		-30.12		0.999	0.0201
Demethoxycurcumin-BSA	298	6.41	5.266	-27.42	109.68	0.999	0.0158
	304	6.66		-28.07		0.998	0.0136
	310	6.96		-28.74		0.999	0.0204

a) SD是 K_a 的标准差

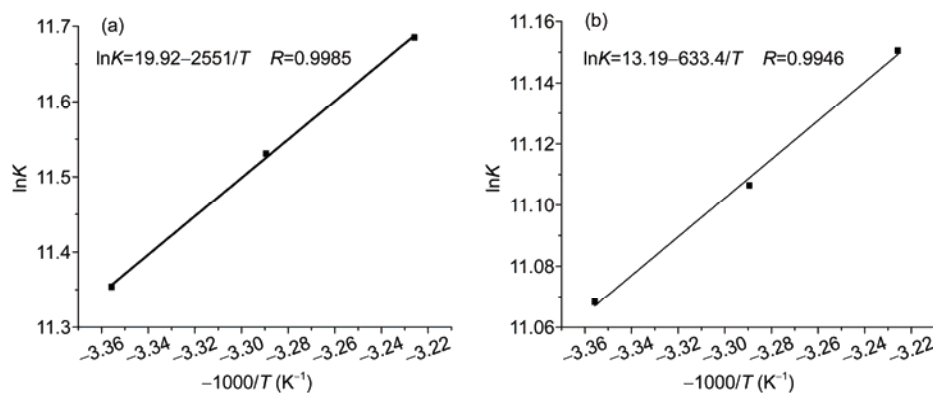


图3 姜黄素(a)及其衍生物去甲氧基姜黄素(b)与BSA相互作用的Van't Hoff关系图. $c(\text{BSA})=1.0 \times 10^{-5} \text{ mol/L}$, pH 7.4

Figure 3 Van't Hoff plots of curcumin-BSA (a) and demethoxycurcumin-BSA (b) system. $c(\text{BSA})=1.0 \times 10^{-5} \text{ mol/L}$, pH 7.4

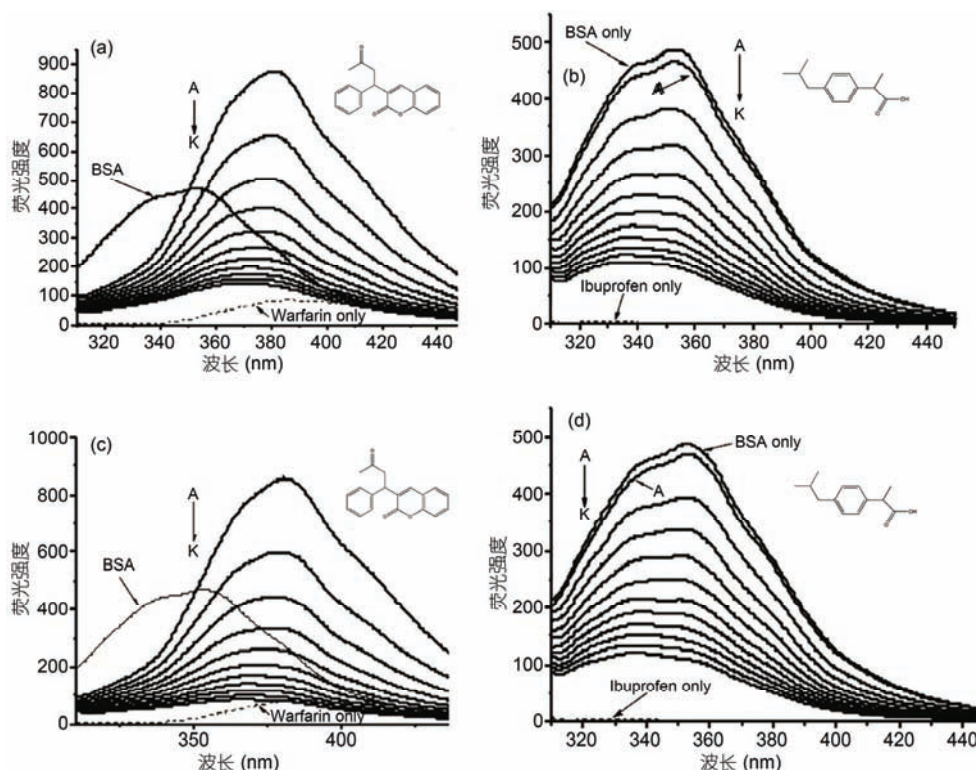


图4 位点探针姜黄素-BSA(a, c)和去甲氧基姜黄素-BSA(b, d)体系的影响. $T=298\text{ K}$, $c(\text{Warfarin})=c(\text{Ibuprofen})=c(\text{BSA})=1\times 10^{-5}\text{ mol/L}$; 从A到K: $c(\text{curcumin})=c(\text{demethoxycurcumin})=0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0\times 10^{-5}\text{ mol/L}$
Figure 4 Effects of site marker to curcumin-BSA (a), (c) and demethoxycurcumin-BSA (b), (d) system. $T=298\text{ K}$, $c(\text{Warfarin})=c(\text{Ibuprofen})=c(\text{BSA})=1\times 10^{-5}\text{ mol/L}$; from A to K: $c(\text{curcumin})=c(\text{demethoxycurcumin})=0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0\times 10^{-5}\text{ mol/L}$

表3 位点竞争结合常数

Table 3 Binding constants of competitive experiments

位点	Curcumin-BSA			Demethoxycurcumin-BSA		
	$K_a(\times 10^4\text{ L/mol})$	R	SD	$K_a(\times 10^4\text{ L/mol})$	R	SD
空白	8.56	0.999	0.0343	6.45	0.999	0.0156
WAR	14.26	0.999	0.0528	16.82	0.998	0.0316
IBP	8.49	0.999	0.0626	7.11	0.998	0.0524

改变的最理想、最常用的方法之一^[30]. 在远紫外区出现蛋白质 α -螺旋结构的2个特征负肩峰谱带(208和222 nm)^[31], 如图5所示. 随着2种药物小分子的逐渐加入, BSA的峰强度在低浓度时增大, 当药物浓度升高到一定程度, BSA的峰强度又开始下降, 但是形状没有太大改变, 表明2种药物分子对BSA的二级结构产生了影响. 表4列出不同摩尔比例时 α -螺旋百分含量, 当药物浓度较低时, α -螺旋含量有所增加, 表明BSA分子的肽链结构在药物的作用下有所折叠; 高浓度时, BSA肽链又发生了伸展, 导致了 α -螺旋含量的降低.

2.5 分子对接

采用Sybyl 8.1软件, 进行分子模拟实验, 来研究2种药物小分子与BSA的结合位点和作用模式^[32]. 分子对接结果显示, 姜黄素和去甲氧基姜黄素都更倾向于结合在BSA的Site I位点, 即华法林位点, 并且在5 Å范围内每种化合物周围都存在不少于10个的氨基酸残基, 如图6(b)和(d)所示. 姜黄素和去甲氧基姜黄素结合位点周围的氨基酸残基主要有13个, 分别为: GLU152, ARG194, ARG198, TRP213, ARG217, LEU237, HIS241, HIS287, ARG435, CYS436, PRO446, CYS447和TYR451. 结合位点周边的氨基酸环境主

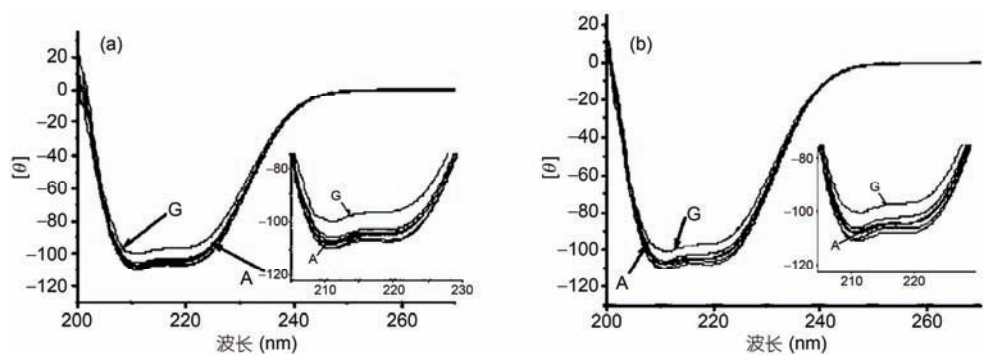


图5 姜黄素(a)和去甲氧基姜黄素(b)与BSA作用的圆二色谱. $c(\text{BSA})=1\times10^{-5}\text{mol/L}$; 从A到G, $c(\text{curcumin})=c(\text{demethoxycurcumin})=0, 0.05, 0.1, 0.2, 1.0, 2.0, 5.0\times10^{-4}\text{mol/L}$
Figure 5 CD spectra of BSA in presence of curcumin (a) and demethoxycurcumin (b). $c(\text{BSA})=1\times10^{-5}\text{mol/L}$; from A to G, $c(\text{curcumin})=c(\text{demethoxycurcumin})=0, 0.05, 0.1, 0.2, 1.0, 2.0, 5.0\times10^{-4}\text{mol/L}$

表4 两种药物小分子与BSA在不同摩尔比例时 α -螺旋含量

Table 4 α -helical content of BSA at different mole ratio between two kinds of drugs and BSA

α -螺旋含量	$n(\text{药物}) : n(\text{BSA})$						
	0:1	0.5:1	1:1	2:1	10:1	20:1	50:1
Curcumin-BSA	46.54%	48.13%	48.22%	46.69%	46.70%	45.76%	41.89%
Demothxycurcum in-BSA	46.64%	48.64%	47.79%	46.56%	46.38%	45.26%	42.19%

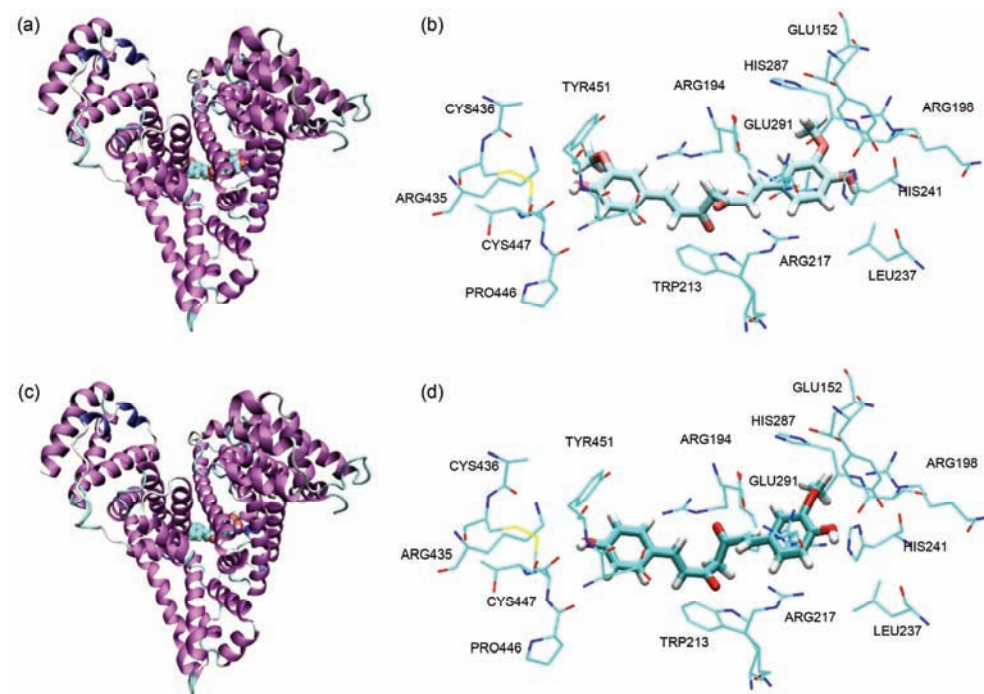


图6 (网络版彩色)姜黄素和去甲氧基姜黄素与BSA的分子对接模拟. (a)和(c)分别是姜黄素和去甲氧基姜黄素在BSA上的结合位点; (b)和(d)分别表示姜黄素和去甲氧基姜黄素与BSA结合位点处的氨基酸残基
Figure 6 (Color online) Computation docking model of the interaction between curcumin/demethoxylcurcumin and BSA. (a), (c) Represents the binding sites of curcumin and demethoxylcurcumin in BSA respectively; (b), (d) represents the amino acid residues around the binding sites for curcumin and demethoxylcurcumin respectively

要为疏水环境, 其中一端的苯环处于TYR451, CYS447, CYS436和PRO446所构成的疏水环境中, 稳定了姜黄素、去甲氧基姜黄素与BSA的结合, 这也和前面热力学数据分析的结果一致. 此外, 姜黄素、去甲氧基姜黄素与TRP213残基的距离均小于5 Å, 表明姜黄素、去甲氧基姜黄素与BSA结合会有效地引起TRP213残基附近微环境的改变, 可能是发生荧光猝灭的机理.

3 结论

姜黄素和去甲氧基姜黄素均能有效猝灭BSA的内源荧光, 猝灭机制属于静态猝灭. 两种药物与BSA的相互作用是一个吉布斯自由能降低的自发过程, 且二者之间的主要作用力为疏水作用力. 两种药物在BSA的主要结合位点均为Site I, 均使BSA的构象发生了改变. 分子模拟的结果与实验结果相一致.

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Thermodynamics of the interaction of curcumin and demothxycurcumin with bovine serum albumin

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Curcumin and its derivatives have a wide range of pharmacological effects such as anti-inflammatory, antioxidant, resisting atherosclerosis, anti-tumor, etc. Binding of curcumin with biological macromolecules can improve the solubility and stability without reducing its biological activity. Herein, we investigated the thermodynamics of interactions of bovine serum albumin (BSA) with curcumin and its derivative demothxycurcumin by fluorescence and circular dichroism (CD) spectroscopy, as well as molecular modeling method under conditions near to physiological. The analysis of fluorescence spectra showed that curcumin and demothxycurcumin could strongly quenching the fluorescence of BSA and the quenching process is a static process. The thermodynamic parameters were calculated by analysis of fluorescence data with Stern-Volmer and Van't Hoff equation. The calculated enthalpy change (ΔH) and entropy change (ΔS) implied that hydrophobic interaction plays a main role in the binding process. Curcumin was more prone to bind with BSA than demothxycurcumin according to the calculated Gibbs free energy change (ΔG). Data obtained by site marker competitive experiments and molecular modeling method indicated that the binding of curcumin and demothxycurcumin to BSA primarily take place in site I. Curcumin and demothxycurcumin mainly interact with nonpolar and aromatic residues of BSA. The distance between curcumin and TRP 213 is less than 5 Å, indicating that the microenvironment around the fluorophore of BSA might be affected. The results of CD spectra demonstrated that the secondary conformation of BSA has been changed after interaction with curcumin and demothxycurcumin. This research will provide valuable information for understanding the action mechanism of curcumin in the body and developing novel drugs based on the structure of curcumin.

curcumin, demothxycurcumin, bovine serum albumin, thermodynamic parameters, fluorescence spectroscopy, circular dichroism spectroscopy, molecular modeling

doi: 10.1360/N972016-00302