2,2'-二乙酰色氨酸甲酯对 Cu^{2+} 的识别传感

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摘要:设计合成了化学传感分子 2,2'-二乙酰色氨酸甲酯(bATM),应用吸收光谱考察了其对金属离子(Cu^{2+},Hg^{2+},Pb^{2+},Zn^{2+},Cd^{2+} 和 Ni^{2+})的响应.结果表明,bATM 的吸收光谱仅对 Cu^{2+} 响应显著,光谱红移 102 nm,溶液由无色转变为黄色,实现了 Cu^{2+} 的高选择性高灵敏裸眼识别.对 Cu^{2+} 的响应线性范围为 $5.0\times10^{-8}\sim1.8\times10^{-5}$ mol/L,检测限为 5.5 nmol/L.初步探讨了传感分子与 Cu^{2+} 的结合模式和光谱传感机制.

关键词:2,2'-二乙酰色氨酸甲酯;Cu^{2+};裸眼识别;吸收光谱
中图分类号:Q 657.39 **文献标志码**:A **文章编号**:0438-0479(2011)06-1023-05

铜是人体必需的微量元素,体内含量仅次于 Fe^{3+} 和 Zn^{2+}.动物体各组织和器官均含有微量铜.许多生命过程中低含量的 Cu^{2+} 参与荷尔蒙、胶原蛋白和弹性蛋白等的生物合成过程[1-2].但过量的 Cu^{2+} 对生物体产生较大毒害,导致门克斯病、威尔逊病和阿尔茨海默病等[3-8].此外,Cu^{2+} 的过量摄入还会造成肠病[9]和婴儿肝损伤[10-11].因此,Cu^{2+} 传感分析研究备受关注.理想的传感体系需具备高的选择性和高的灵敏度,同时能快速在线响应,并具良好的生物相容性[12-21].

色氨酸作为组成蛋白质的基本单元,是人体必须氨基酸.一定条件下色氨酸可被氧化偶联为二色氨酸偶联结构单元,该结构单元于蛋白质各级结构构建中发挥着重要作用[22-29].因其显著的光学性质和良好的生物相容性,以二色氨酸偶联结构单元作为化学传感分子骨架构建传感体系应当具有较大的潜力.本文设计合成了含有多肽 γ-回旋和 β-折叠二级结构基本单元的 2,2'-二乙酰色氨酸甲酯(bATM,图 1)作为 Cu^{2+} 的新型光谱传感分子,实现了乙腈中 Cu^{2+} 的高灵敏度、高选择性识别和光谱传感.

1 实验部分

1.1 仪器与试剂

Bruker AV 400 MHz 核磁共振波普仪(TMS 为内标);Micromass-LCT 高分辨质谱仪;Carlo Erba Instruments/CE Instruments EA/MA1110 元素分析仪;Thermo Evolution 300 紫外-可见吸收光谱仪;Hitachi F-4500 荧光光谱仪;JASCO Corporation J-810 圆二色光谱仪.

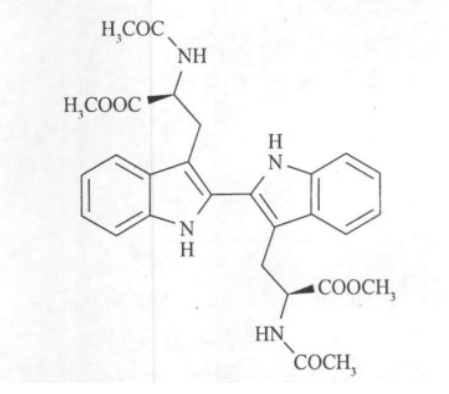

图 1 bATM 的分子结构
Fig. 1 Molecular structure of bATM

合成用试剂均为国药化学试剂公司产品.光谱传感实验所用阳离子为其高氯酸盐(分析纯),所用有机溶剂均重蒸馏纯化.实验用水为三次重蒸去离子水.

1.2 bATM 合成与表征

5.0 g 色氨酸溶于甲醇,于冰盐浴中搅拌并缓慢滴加 5.0 mL 氯化亚砜,加热回流 6 h 得色氨酸甲酯粗产品.将经由乙酸乙酯重结晶纯化后的色氨酸甲酯 3.0 g 分散于二氯甲烷中,吡啶存在下与 1.5 mL 乙酸酐室温搅拌反应 6 h,然后依次用稀盐酸和水洗涤,旋转蒸发除去二氯甲烷后得到淡黄色固体乙酰色氨酸甲酯.

bATM 的合成参照文献[30-34].1.0 g 乙酰色氨

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酸甲酯溶解于三氟乙酸中,氮气除氧并室温搅拌 9 h. 加入三氯甲烷稀释后旋转蒸发去除溶剂,将得到的固体干燥后再溶于 3.0 mL 1,4-二氧六环,加入 0.23 g 2,3-二氯-5,6-二氰基-1,4-苯醌(DDQ),室温搅拌 5 h. 反应完毕后加入三氯甲烷,用饱和碳酸氢钠溶液反复洗涤至水相澄清. 将有机相旋转蒸发,用三氯甲烷重结晶得到白色粉末 bATM.

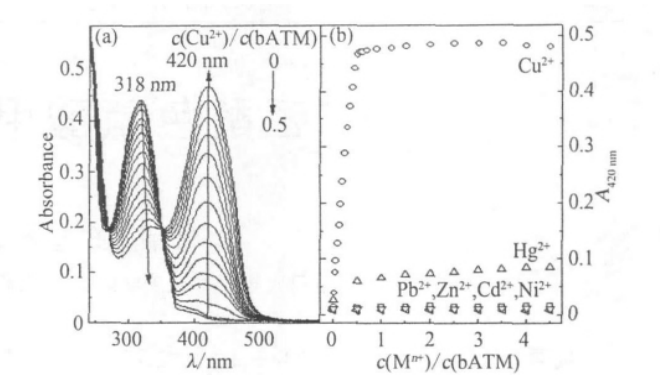
产品经 ^{1}H-NMR、^{13}C-NMR、HR-MS(ESI) 和元素分析表征. ^{1}H-NMR(400 MHz,DMSO-d_6):δ 11.16(s,2H),8.08 (d,J = 8.0 Hz,2H),7.57(d,J = 8.0 Hz,2H),7.38(d,J = 8.0 Hz,2H),7.14(t,J = 8.0 Hz,2H),7.05(t,J = 8.0 Hz,2H),4.49(d,J = 8.0 Hz,2H),3.30(s,6H),3.16(m,4H),1.66(s,6H); ^{13}C-NMR(400MHz,DMSO-d_6):δ 172.13,169.20,136.25,127.89,127.72,125.26,121.68,118.81,118.58,111.29,108.89,52.87,51.57,27.17,22.12; HR-MS(正离子模式)理论值[M + H]$^+$:m/z 519.224 4;实测值为:519.225 6. 元素分析理论值为 $C_{28}H_{30}N_4O_6$:C,64.85;H,5.83;N,10.80;O,18.51;实测值为 C,64.80;H,6.134;N,10.51;O,18.556.

2 结果与讨论

2.1 乙腈中传感分子吸收光谱对金属离子的响应

乙腈中传感分子 bATM 的最大吸收位于 318 nm,摩尔吸光系数为 1.45×10^4 L/(mol · cm),说明 bATM 的吸收源自生色团吲哚的 π-π^* 电子跃迁. 图 2 为乙腈中 bATM 的吸收光谱随 Cu^{2+} 浓度的变化轨迹. 随 Cu^{2+} 浓度提高,bATM 的吸收光谱于 420 nm 处出现新的吸收峰且吸光度逐渐增强,而 318 nm 处吸收峰逐渐减弱,光谱红移达 102 nm. 同时于 270 和 348 nm 处观察到 2 个清晰的等吸收点,表明受体分子 bATM 与 Cu^{2+} 形成了具有确定组成的配合物. 光谱滴定实验表明 bATM 的吸光度与 Cu^{2+} 浓度呈良好的线性关系(R^2 = 0.995 1),线性范围为 5.0×10^{-8} ~ 1.8×10^{-5} mol/L,检测限($3\sigma/k$,n = 11)低至 5.5 nmol/L. 说明 bATM 对 Cu^{2+} 具有高的检测灵敏度. 因光谱的显著红移,Cu^{2+} 加入后 bATM 的乙腈溶液由无色转变为黄色,可实现 Cu^{2+} 的裸眼检测.

相同条件下考察了其他 5 种常见过渡金属离子 Hg^{2+},Pb^{2+},Zn^{2+},Cd^{2+} 和 Ni^{2+} 对 bATM 吸收光谱的影响. 实验表明,除 Hg^{2+} 能引起微弱的光谱变化外,Pb^{2+},Zn^{2+},Cd^{2+} 和 Ni^{2+} 4种金属离子均未导致bATM



c(bATM) = 5.0×10^{-5} mol/L,$c(Cu^{2+})$ = 0 ~ 2.5×10^{-5} mol/L.

图 2 乙腈中 Cu^{2+} 存在时 bATM 的吸收光谱(a)和 bATM 于 420 nm 处吸光度与金属离子浓度关系曲线(b)

Fig. 2 Absorption spectra of bATM in acetonitrile in the presence of Cu^{2+} ion (a) and plots of absorbance of bATM at 420 nm against metal ion concentration (b)

吸收光谱的任何变化,说明 bATM 的吸收光谱对 Cu^{2+} 具有高的响应选择性.

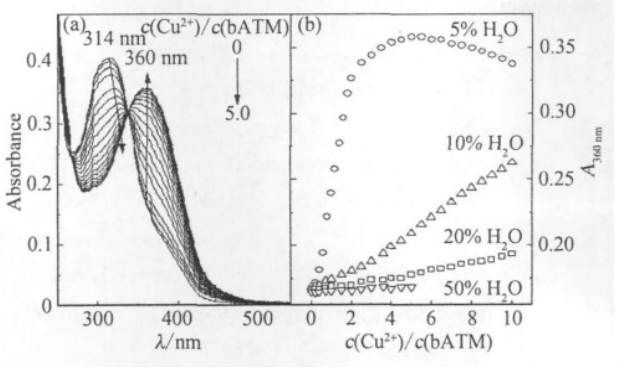
2.2 乙腈-水混合溶剂中 bATM 对 Cu^{2+} 的识别传感

考察了乙腈-水(体积比 95 ∶ 5 ~ 50 ∶ 50)混合溶剂中 bATM 吸收光谱对 Cu^{2+} 的识别响应. 乙腈-水(体积比 95 ∶ 5)混合溶剂中 bATM 的最大吸收峰由纯乙腈中的 318 nm 蓝移至 314 nm,传感分子与水之间的氢键作用可能是前者光谱蓝移的原因之一. 与纯乙腈中的光谱变化类似,Cu^{2+} 的引入使 bATM 于 314 nm 处的吸光度逐渐减小,360 nm 处出现新的吸收峰且吸光度随 Cu^{2+} 浓度增大逐渐增强(图 3(a)),同时于 268 和 340 nm 处观察到 2 个清晰的等吸收点. 随着混合溶剂中水含量增加,bATM 对 Cu^{2+} 的光谱响应逐渐减弱(图 3(b)),可能是水对 Cu^{2+} 的强溶剂化作用所致. 该结果说明传感分子对 Cu^{2+} 的选择性光谱传感可拓展至含水体系.

2.3 识别模式与机理

乙腈中 bATM 的吸收光谱 Cu^{2+} 滴定结果显示(图 2(b)),Cu^{2+} 浓度达到 bATM 的 0.5 倍量时,bATM 的 420 nm 处吸光度达到饱和,表明 Cu^{2+} 与 bATM 形成了 1 ∶ 2 计量比的配合物. 等摩尔连续变化法(Job plot)进一步说明 Cu^{2+} 与传感分子的结合计量比为 1 ∶ 2(图 4).

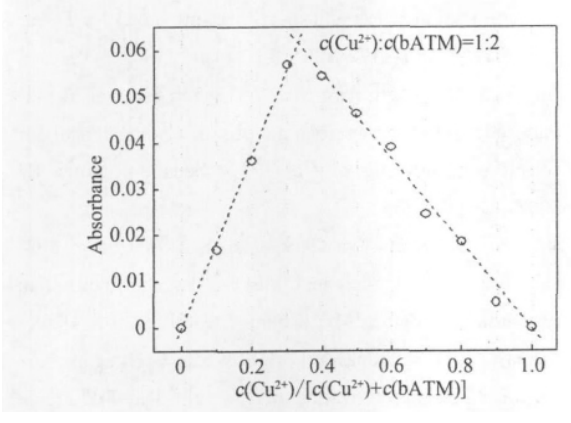
圆二色(CD)光谱是考察分子构象变化的重要手段之一. 因 bATM 分子含有手性色氨酸结构单元而具有手性中心,考察乙腈中 bATM 的 CD 光谱对 Cu^{2+} 的响应应可以指示bATM与Cu^{2+}配位过程中的构象



(a) $c(bATM)=5.0\times10^{-5}$ mol/L,
$c(Cu^{2+})=0\sim2.5\times10^{-4}$ mol/L.

图 3　乙腈-水(体积比 95∶5)混合溶剂中 Cu^{2+} 存在下 bATM 的吸收光谱(a)和乙腈-水混合溶剂中 bATM 于 360 nm 处吸光度随 Cu^{2+} 浓度的变化关系曲线(b)

Fig. 3　Absorption spectra titration of bATM in 95∶5 acetonitrile-water (by vol.) binary aqueous solution in the presence of Cu^{2+} (a) and plots of absorbance of bATM at 360 nm against Cu^{2+} concentration in acetonitrile-water binary solvents of varying water volume fraction (b)

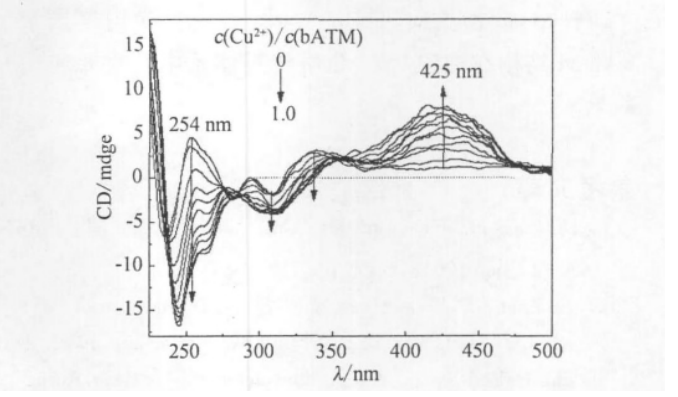


$c(Cu^{2+})+c(bATM)=1.0\times10^{-5}$ mol/L.

图 4　乙腈中 bATM 与 Cu^{2+} 相互作用的 Job 曲线

Fig. 4　Job plot for the interaction of bATM with Cu^{2+} in acetonitrile

变化. 图 5 表明乙腈中 bATM 的 CD 光谱于 236,280 和 310 nm 处呈现负的 Cotton 效应,254 和 338 nm 处呈现正的 Cotton 效应. 随着 Cu^{2+} 加入 310 nm 处 CD 信号逐渐增强,338 nm 处 CD 信号逐渐减弱,254 nm 处正 Cotton 效应则翻转成为负的 Cotton 效应,并于 425 nm 处出现新的 CD 信号峰,后者与吸收光谱于 420 nm 出现新的吸收峰(图 2)相对应. 254 nm 处 CD 信号的翻转和 425 nm 处 CD 信号的显著增强均说明 bATM 与 Cu^{2+} 配位过程中的构象变化,使 bATM 的二吲哚环结构平面性较未配位前更强. 量子力学计算



$c(bATM)=1.0\times10^{-4}$ mol/L, $c(Cu^{2+})=0\sim1.0\times10^{-4}$ mol/L.

图 5　乙腈中 Cu^{2+} 存在下 bATM 的 CD 光谱

Fig. 5　Circular dichroism spectra of bATM in acetonitrile in the presence of Cu^{2+}

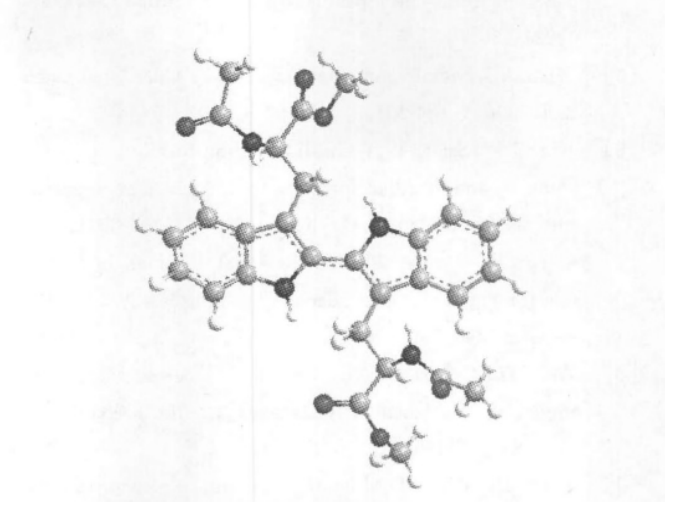


图 6　bATM 的最低能量结构(B3LYP/6-31G*)

Fig. 6　Energy-minimized (B3LYP/6-31G*) structure of bATM

(B3LYP/6-31G*)得到的 bATM 分子结构示于图 6,也指示 bATM 分子中 2 个吲哚环呈近平面结构,手性基团处于反式构型. Cu^{2+} 与吲哚生色团的氮原子配位而使联吲哚基团构象翻转,导致 bATM 的 CD 信号的显著变化.

3　结　论

本文设计合成了 bATM 传感分子,考察了乙腈和乙腈-水(体积比 95∶5～50∶50)混合溶剂中其吸收光谱对 Cu^{2+} 的识别响应. bATM 吸收光谱显示出对 Cu^{2+} 具有高灵敏、高选择性的响应. 摩尔比法和等摩尔连续变化法揭示 bATM 与 Cu^{2+} 以 2∶1 的计量比配位,CD 光谱和量化计算结果表明 Cu^{2+} 与 bATM 配位后使受体分子中联吲哚结构发生构象翻转的同时分

子刚性增强. bATM 与 Cu^{2+} 的独特配位作用展示了 bATM 结构在构建 Cu^{2+} 化学传感体系中的重要应用前景.

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Methyl 2,2′-bis(*N*-acetyltryptophan) as a Highly Sensitive and Selective Chemosensor for Cu^{2+}

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Abstract: A 2,2′-bistryptophan derivative (bATM) was designed as a selective and sensitive chemosensor for Cu^{2+} among heavy metal ions (Cu^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+} and Ni^{2+}) in acetonitrile and water-acetonitrile binary solvents. Absorption spectrum of bATM in acetonitrile peaked at 320 nm was found to exhibit a huge red-shift to 420 nm in the presence of Cu^{2+}, together with the appearance of two isosbestic points at 270 nm and 350 nm. In water-acetonitrile binary solvents however the absorption spectral evolution becomes weaker and the spectral variation is less with increasing water content, suggesting that the hydration of Cu^{2+} plays a role. The red-shift in water-acetonitrile binary solvents was found to be smaller too, now from 314 nm to 360 nm, despite the observation of two isosbestic points. In acetonitrile the absorbance of bATM at 420 nm is linear to Cu^{2+} concentration over 5.0×10^{-8} mol/L to 1.8×10^{-5} mol/L, with a detection limit of 5.5 nmol/L ($3\sigma/k$). Job plot indicates that bATM coordinates with Cu^{2+} in a 2∶1 stoichiometry. CD spectral titrations in acetonitrile suggest a conformation change in bATM upon coordinating Cu^{2+} that it takes a *cis*-conformation in terms of its two tryptophan moieties and becomes more planar in the Cu^{2+}-complex. Although detailed sensing mechanism remains to be clarified, the observed high sensitivity and selectivity of bATM in its absorption spectral response toward Cu^{2+} suggests that the structural framework of bATM deserves further effort for developing sophisticated chemosensors for transition metal ions, for example by modifying its structure in the amino acid moiety.

Key words: methyl 2,2′-bis(*N*-acetyltryptophan); Cu^{2+}; naked-eye detection; absorption