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nm 荧光峰的强度, K_{sv} 为猝灭常数, $[In]$ 为吲哚季铵盐浓度。以 F_0/F 对 $[In]$ 作图可得直线 (见图 5), 由斜率可得 K_{sv} 为 $5116 \times 10^4 \text{ dm}^3 \cdot \text{mol}^{-1}$ 。

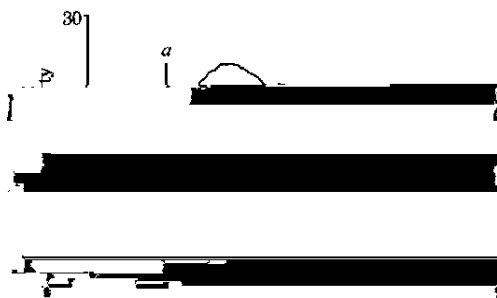


Fig14 Fluorescence spectra of BSA ($1 \times 10^{-6} \text{ mol} \cdot \text{dm}^{-3}$) in the presence of compound 6: a, 0; b, $2 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$; c, $2 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$; d, $4 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$; e, $6 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$; f, $8 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$, $E_x = 280 \text{ nm}$

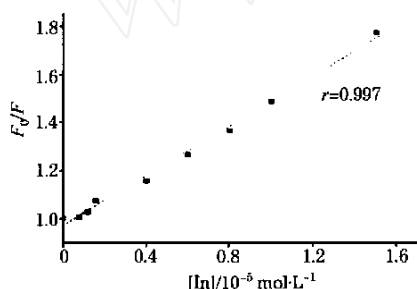


Fig15 Stern Volmer plot of F_0/F vs $[In]$

为验证其猝灭类型, 将此过程按动态过程处理, 则

$$K_{sv} = k_q \tau_0 \quad (2)$$

其中, k_q 为双分子猝灭过程速率常数, τ_0 为猝灭体不存在时荧光分子平均寿命。由于生物大分子荧光寿命约为 $10^{-8} \text{ s}^{[16]}$, 所以由方程 (2) 可得 k_q 为 $5116 \times 10^{12} \text{ dm}^3 \cdot (\text{mol} \cdot \text{s})^{-1}$ 。而各类猝灭剂对生物分子的最大碰撞猝灭过程速率常数为 $210 \times 10^{10} \text{ dm}^3 \cdot (\text{mol} \cdot \text{s})^{-1}^{[16]}$, 所以反证以上猝灭为静态猝灭。

213 结合数和结合常数

对于静态猝灭过程, 荧光强度与猝灭剂的关系可由荧光分子与猝灭剂分子之间的结合常数表达式推导出^[3]。设生物大分子 BSA 和 3H2 吲哚季铵盐 In 有 n 个相同且独立的结合位点, 则两者间的猝灭反应可表示为



其相应的结合常数 K_a 为:

$$K_a = \frac{[In_nBSA]}{[In]^n [BSA]} \quad (4)$$

式中 $[BSA]$ 为游离荧光体浓度, $[In_nBSA]$ 是配合物浓度, 若荧光体总浓度为 $[BSA]_0$, 则 $[BSA]_0 = [In_nBSA] + [BSA]$, 代入 (4) 式得:

$$K_a = \frac{[BSA]_0 - [BSA]}{[In]^n [BSA]} \quad (5)$$

在静态猝灭中, 荧光体系的荧光强度 F 与其游离浓度成正比 (所生成的配合物是非荧光性的)

$$\frac{[BSA]}{[BSA]_0} = \frac{F}{F_0} \quad (6)$$

由 (5) 和 (6), 可得

$$\lg \frac{(F_0 - F)}{F} = \lg K_a + n \lg [In] \quad (7)$$

当加入的 3H2 吲哚季铵盐足够多时, 加入的 3H2 吲哚季铵盐远远大于结合的 3H2 吲哚季铵盐, 即 $[In]_0 \gg [In_nBSA]$ 时, 近似地用 $[In]_0$ 来代替 $[In]$ (见图 6)。对其中线性部分拟合, 求得 3H2 吲哚季铵盐与白蛋白分子的结合常数 K_a 及结合位点数 n $K_a = 11995 \times 10^5 \text{ dm}^3 \cdot \text{mol}^{-1}$, $n = 1112$ 。由于低浓度不满足 $[In]_0 \gg [In_nBSA]$, 所以偏离直线较大。

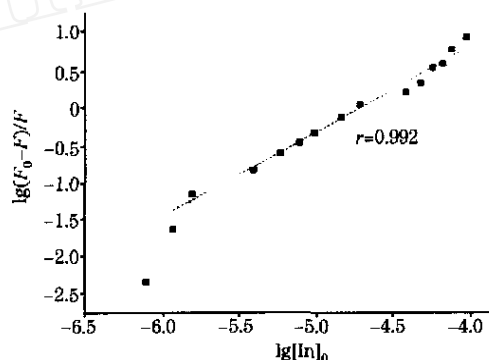


Fig16 Plot of $\lg(F_0 - F)/F$ vs $\lg[In]_0$

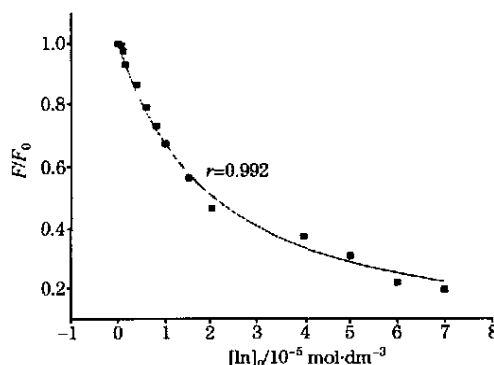


Fig17 Langmuir plot of F/F_0 vs $[In]_0$

对 3H2 吲哚季铵盐分子与牛血清蛋白的作用过程, 用 Langmuir 吸附等温式处理, 则覆盖率 θ 为

$$\theta = \frac{\frac{k_1}{k_{-1}} [In]_0}{1 + \frac{k_1}{k_{-1}} [In]_0} = \frac{K [In]_0}{1 + K [In]_0} \quad (8)$$

式中 k_1 , k_{-1} , K 分别为吸附、解吸速率常数和吸附平衡常数。覆盖率 θ 应为被结合的蛋白分子占总蛋白的比率, 即

$$\theta = \frac{[BSA]_0 - [BSA]}{[BSA]_0} = \frac{F_0 - F}{F_0} \quad (9)$$

由公式(8)和(9)可推得

$$\frac{F}{F_0} = \frac{1}{1 + K[\text{In}]_0} \quad (10)$$

根据公式(10)拟合数据(见图7),得到吸附常数 $K = 4184 \times 10^4 \text{ dm}^3 \cdot \text{mol}^{-1}$ 。此值与 K_a 有一定偏差,可能是由于所用的模

型不同所致。

关于 3H2吲哚以及其他几种吲哚季铵盐与蛋白质的作用正在研究之中,希望通过这些研究,能够得到更多关于此类连有季铵盐的荧光探针分子与蛋白质作用的信息。

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Synthesis of Substituted 3H2Indole Quaternary Ammonium Salt and Study on Its Interaction with Bovine Serum Albumin by Fluorescence

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Abstract A substituted 3H2indole quaternary ammonium molecule was designed and synthesized using hexamethylphosphoramide (HMPA) as a solvent. The products were purified and characterized by IR, ¹H2NMR, MS and elemental analysis. The binding reaction of this compound with bovine serum albumin (BSA) in aqueous solution was studied using fluorescence. Their binding constant is $K_a = 11995 \times 10^5 \text{ dm}^3 \cdot \text{mol}^{-1}$ and the binding site number is $n = 1112$. It is confirmed that the combination is a single static quenching process.

Keywords Substituted 3H2indole quaternary ammonium molecule; Synthesis; BSA; Fluorescence

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