温度对铽(III)-转铁蛋白溶液构象的影响

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摘要 在pH 7.4, 0.01 mol/L N-2-羟乙基哌嗪-N'-2-乙磺酸(Hepes)条件下,铽(III)与N, N'-二(2-羟苄基)乙二胺-N, N'-二乙酸(HBED)结合并发生交换 相互作用使铽(III)荧光增强10~4倍,通过监测铽(III)545 nm荧光强度的变化 测定了Tb-HBED配合物的条件稳定常数是lgK = 14.30 ± 0.49; Tb-HBED配合物中配体、铽(III)荧光强度均随着温度的升高而降低。在pH 7.4, 0.01 mol/L Hepes条件下,Tb_N-apoTf-Tb_C配合物中蛋白质的荧光强度随着温度的升高而降 低,而能量受体铽(III)的荧光强度随着温度的升高而增强,主要源于铽(III)与螺旋5色氨酸残基间的无辐射能量转移; 当温度由0℃上升到55℃时,平均能量 转移效率AE值增加了29%,给体、受体间距离R有约4.2%的减小,温度变化引起Tb_N-apoTf-Tb_C配合物大的构象变化; 铽(III)与人血清脱铁转铁蛋白的结合使蛋白质的变性温度降低。同样条件下,Tb_N-apoOTf-Tb_C配合物与Tb_N-apoTf- Tb_C配合物有所不同,虽然能量给体的荧光强度随着温度的增加而减小,但铽(III)荧光强度没有明显的增强; 铽(III)

对蛋白质的变性温度几乎没有影响。 关键词 铽 构象 荧光分光光度法 乙二胺 P 乙酸 P 蛋白质

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Effect of Temperature on the Conformation of Diterbium(III) Transferrin

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Abstract In 0.01 mol/L N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (Hepes), pH 7.4, the binding of N,N'-bis(2-hydroxybenzyl) ethylenedinitrilo-N,N'-diacetic acid (HBED) with Tb(III) was monitored by fluorescence spectra. As the exchange interaction between HBED and Tb(III), the Tb(III) emission intensity at 545 nm is greatly enhanced. The conditional equilibrium constant for Tb-HBED is lgK = 14.30 ± 0. 49. HBED emission intensity at 311 nm or Tb(III) at 545 nm decreases as the temperature rises. For the complex of Tb(III) ions with apotransferrin, Tb_N-apoTf-Tb_C, the protein emission intensity at 338 nm decreases as the temperature rises, while the Tb(III) emission intensity at 549 nm increases gradually with increased temperature. The experimental phenomenon may be resulted from energy transfer between Tb(III), the acceptor, and Trp residue in helix 5, the donor. According to Forster type dipole-dipole radiationless energy transfer, the average efficiency of energy transfer from Trp to Tb(III) increases 29% over 55 °C temperature range, this represents a 4.2% decreases in donor-acceptor distance (R). The denaturation of the protein begins at 60 °C after Tb (III) ions bind to apotransferrin. For the complex of Tb(III) ions with apoovotransferrin, Tb_N-apoOTf-Tb_C, there is a decrease in protein emission intensity at 336 nm as temperature rises, but there is no significent increase in Tb(III) emission intensity at 549 nm. Tb(III) binding to apoovotransferrin has no effect on the denaturation point of the protein. It means that there is different conformational behavior for Tb_N-apoTf-Tb_C and Tb_N-apoOTf-Tb_C.

Key words TERBIUM CONFORMATION FLUOROSPECTROPHOTOMETRY ETHANEDIAMINE ACETIC ACID P PROTEIN

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