钙(Ⅱ)对抗凝血因子Ⅱ与活化凝血因子X结合反应的影响

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摘要 皖南尖吻蝮蛇毒抗凝血因子II(ACFII)不具有酶的活性,通过与活化凝血因子X(FXa)结合成1:1的复合物来延长凝血时间。用高效液相色谱方法,发现ACFII与FXa的结合反应依赖于Ca(II)浓度,ACFII与FXa的最大结合反应所需要的CaII浓度约为1×10^-3mol/L。平衡透析的结果表明,ACFII分子中有两个不同亲和性的Ca(II)结合位点,表观结合常数分别为(1.1±0.3)×10^5L/mol和(1.7±0.4)×10^4L/mol,ACFII与2个Ca(II)结合所需要的Ca(II)浓度也约为1×10^-3mol/L。Ca(II)对ACFII的荧光有增强效应,其量大荧光增强所需要的Ca(II)浓度也约为1×10^-3mol/L。由此推测ACFII结合上2个Ca(II)可能性是其与FXa结合的前提条件。

关键词  $\underline{\text{结合蛋白}}$   $\underline{\text{抗凝血因子II}}$   $\underline{\text{活化凝血因子X}}$  <u>钙离子</u>

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# The effect of calcium ( $\rm II$ ) on the binding of anticoagulation fator $\rm II$ with activate coagulation factor $\rm X$

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**Abstract** Anticoagulation factor II(ACFII) from the venom of Agkistrodon acutus is a non-enzymatic anticoagulant and forms a 1:1 complex with activated coagulation factor X(FXa), to thereby prolong clotting time. It has been discovered by HPLC that the binding of ACF II with FXa is dependent on the concentration of Ca(II) and the maximal binding of ACF II to FXa occurs at concentration of Ca(II) of about  $1 \times 10^{\circ}$ -3mol/L. The binding of Ca(II) to ACF II is analyzed by equilibrium dialysis and two Ca(II)-binding sites with different affinities are identified. At pH 8.0, the apparent association constants k1 and k2 values for these two sites are  $(1.1\pm0.3)\times10^{\circ}$ 5L/mol and  $(1.7\pm0.4)\times10^{\circ}$ 4L/mol, respectively. It is evident from the observation of Ca(II) induced changes in the intrinsic fluorescence of ACF II that ACF II undergoes a conformational change upon binding of Ca(II). The occupation of both Ca(II)-binding sites in ACF II requires a concentration of Ca(II) of about  $1\times10^{\circ}$ -3mol/L, which is equal to the effective concentration of Ca(II) required for the maximal binding of ACF II to FXa and for the maximal Ca(II)-induced enhancement of emission fluorescence of ACF II. It can be deduced, from these results, that the occupation of both Ca(II)-binding sites in ACF II with Ca(II) and subsequent conformational rearrangement might be essential for the binding of ACF II with FXa.

Key words CONJUGATED PROTEIN CALCIUM ION

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