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Study of complex formation between calmodulin and two isoforms of rabbit elongation factor eEF1-A by SAXS method

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Introduction

The main function of higher eukaryotic translation elongation factor eEF1A is the delivery of correct aminoacyl-tRNA to the A site of mRNA-programmed ribosome in translation cycles [1]. Besides, eEF1A is involved in other cellular processes like carcinogenesis and apoptosis and can interact with the large number of non-translational ligands in cell. There are two tissue and development-specific isoforms of eEF1A, which are 97% homologous. Importantly, despite on strong similarity of amino acid sequences, the isoforms appear to differ in some functions. It was found that the appearance of eEF1A2 in non-inherent tissues can be coupled to the cancer development The structural peculiarities determining the cancer specificity of eEF1A2 remain unknown. Thus, minor changes in amino acid sequence cause the functional variation. We reasoned that the background for the functional difference of eEF1A1 and eEF1A2 might lay in the changes of spatial structure of the proteins. These changes may influence on the binding of different ligands.

Here we present the comparison of conformations of complex of calmodulin with rabbit eEF1A1 from liver and eEF1A2 from muscle.

Experimental

eEF-1A were purified from rabbit liver and muscle using a combination of gel filtration, ion-exchange, and hydroxyapatite chromatographies in the presence of 20% glycerol and 20mkM GDP. The buffer conditions are: 30mM Tris-HCl (pH7.5), 10mM KCl, 1mM $MgCl_2$, 10mM $CaCl_2$, 6mM β-mercaptoethanol, 20mkM GDP, 20%(v/v) glycerol. Protein concentrations were 2-3 mg/ml. Molar ratio 1:1 of calmodulin and eEF1A have been studied. Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q=0.008-0.2 $Å^{-1}$.

Results

It was found that Guinier plot for both complexes of eEF1A demonstrates the nonlinear behavior at the very small scattering angles reflecting some association of protein which is more pronounced in the case of eEF1A1. It should be stressed that there is the shift of Guinier plot in absolute scale upward in both cases pointing out the formation of complex. More clearly one can see the conformations of complexes from Kratky plot which is less sensitive to a protein association. In Fig.1 the Kratky plots for complex of eEF1A1 and eEF1A2 with calmodulin are presented in the comparison with those for the unliganded ones . One can see the shift of maximum on the plot to smaller scattering angles indicating the formation of complexes with higher dimensions. Also one can see more expressed changes of scattering pattern in the case of eEF1A1. More strong interaction of eEF1A1 with calmodulin was also noticed by us in the sedimentation experiments where the sedimentation coefficient was practically the same at the presence or absence of calmodulin for eEF1A2. Thus, there is noticeable difference in the kind of interaction of two isoforms of EF1A with calmodulin.

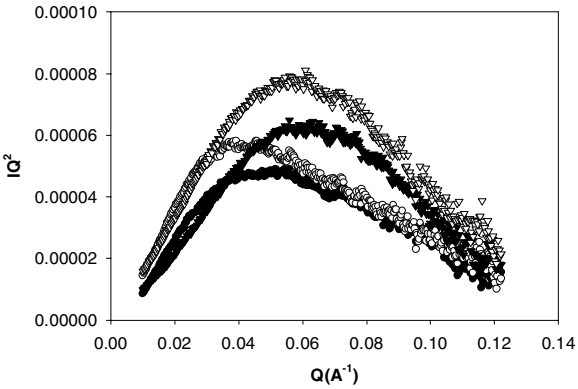

Fig.1 Kratky plot for eEF1A1(•), eEF1A2(▼), eEF1A1+calmodulin (**o**), eEF1A2+calmodulin (∇).

References

1) Negrutskii & El'skaya , *PNAS*, **60**,47, 1998

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