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CALCYCLIN-LIKE PROTEIN FROM EHRLICH ASCITES TUMOUR CELLS. Ca^{2+} AND Zn^{2+} BINDING, DISTRIBUTION AND TARGET PROTEIN *

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The calcyclin-like protein from Ehrlich ascites tumour cells is a 10.5 kDa heat stable protein, which binds two Ca^{2+} ions each with different affinity. Upon Ca^{2+} binding, the protein changes its conformation exposing hydrophobic regions. In this conformation it is able to interact with fluphenazine and with a 36 kDa protein immunologically similar to mammalian calpactin. Calcyclin-like protein binds Zn^{2+} and forms dimers like other members of the S-100 protein family. The calcyclin-like protein is present in several mouse tissues such as stomach, skeletal muscle, heart, spleen, lung and kidney, but seems to be absent from brain, intestine and liver as well as from some tumourigenic cells lines.

We have purified to homogeneity a 10.5 kDa Ca^{2+}-binding protein from Ehrlich ascites tumour (EAT) cells [1]. This protein differs from the S-100 protein, calbindin 9K, parvalbumin and oncomodulin in electrophoretic mobility in SDS-or in urea-polyacrylamide gels, amino acid composition, and lack of cross-reactivity with the antibodies specific to these Ca^{2+}-binding proteins. Recently, we found that the partial amino acid sequence of the 10.5 kDa Ca^{2+}-binding protein from EAT cells is homologous to that of human calcyclin, the growth factor-inducible gene product [2, 3], and therefore we called the mouse protein a calcyclin-like protein [4]. It has been suggested that calcyclin protein is involved in the control of cell proliferation and may bind Ca^{2+}, as deduced from nucleotide sequence of the gene. To our best knowledge nobody has so far studied the protein itself.

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MATERIALS AND METHODS

Calcyclin was purified from Ehrlich ascites tumour cells according to Kuźnicki & Filipek [1]. Polyacrylamide gel electrophoresis in the presence of SDS, immunoblotting, $^{45}Ca^{2+}$ and $^{65}Zn^{2+}$-binding on nitrocelluose were performed as described in Kuźnicki *et al.* [4].

RESULTS AND DISCUSSION

Using gel filtration of the calcyclin-like protein from Ehrlich ascites tumour cells, in the presence of 3×10^{-5} M $^{45}CaCl_2$, we were able to show that this protein binds one Ca^{2+} ion. Ca^{2+}-binding (by the calcyclin-like protein) was directly visualised when the protein was subjected to SDS-PAGE, blotted onto nitrocellulose and incubated with $^{45}Ca^{2+}$.

The binding of Ca^{2+} ions to the calcyclin-like protein was studied by several indirect methods. For example, tyrosine fluorescence intensity of the protein reversibly increased by 18% upon Ca^{2+} binding. The titration curve of fluorescence intensity, plotted against the molar ratio of Ca^{2+} added to the calcyclin-like protein, suggested the existence of two Ca^{2+} binding sites per molecule. Since gel filtration indicated only one Ca^{2+}-binding site, we concluded that there are two binding sites which differ in their affinity for Ca^{2+} [1].

The calcyclin-like protein from EAT cells changes its conformation upon Ca^{2+} binding as indicated by Ca^{2+}-dependent changes of: tyrosine fluorescence intensity, UV absorbance spectrum, mobility in urea-PAGE and hydrophobicity. The changes in exposure of hydrophobic domain(s) have been demonstrated by Ca^{2+}-dependent binding to phenyl-Sepharose (used for purification of the protein) and to fluphenazine-Sepharose; calcyclin-like protein also binds Zn^{2+} (Filipek, Kuźnicki & Heizmann, *FEBS Lett.*, in press). Zinc binding sites seem to be different from calcium binding sites since: preincubation with Ca^{2+} has not effect on Zn^{2+} binding: Ca^{2+}, but not Zn^{2+}, increase tyrosine fluorescence intensity; binding of Zn^{2+} does not block conformational changes induced by Ca^{2+}. These data suggest that both cations: Ca^{2+} and Zn^{2+} may be relevant for the biological activity of calcyclin.

Since the calcyclin-like protein was purified from tumour cells it was interesting to know whether it is a protein specific for tumour cells or whether it is also present in normal cells and tissues. This problem was studied using three methods: Immunoblotting with a polyclonal, affinity purified antibody against calcyclin-like protein, purification of a protein from normal mouse tissues with similar properties, Northern blotting with a full length calcyclin cDNA as a probe.

A 10.5 kDa immunoreactive protein is present in low ionic strength extracts from mouse spleen, heart, skeletal muscle, stomach and cultured rat fibroblasts.

This protein has also been found in extracts from lung and kidney, but no positive reaction was detected in extracts from brain, intestine and liver [5].

A calcyclin-like protein was also purified from mouse stomach (using the method developed for the purification of this protein from EAT cells) and was found to be identical with the EAT protein in respect of molecular weight, isoelectric point and Ca^{2+}-dependent conformational changes [5]. These results indicate that the calcyclin-like protein is present not only in tumour cells, but also in normal cells and tissues. This conclusion was confirmed by Northern blot analysis with the use of a full length calcyclin cDNA. The hybridization data revealed a high level of calcyclin mRNA in EAT cells, much lower amounts in stomach and very little in other mouse organs studied [5].

We have also been looking for a target protein for the calcyclin-like protein using affinity chromatography. A protein fraction from EAT cells was applied onto the calcyclin-Sepharose column in the presence of Ca^{2+}. The unbound proteins were eluted in buffers containing Ca^{2+} and high concentrations of NaCl. The proteins which bound to calcyclin in the presence of Ca^{2+} were subsequently eluted with the buffer containing EGTA. The major protein band enriched by this method had an apparent molecular mass of about 36 kDa and was found to react with an antibody against mammalian calpactin (p36-p11 complex) in immunoblots. The results indicate that in EAT cells one of the possible target proteins of the calcyclin-like protein is immunologically similar to the members of the calpactin-lipocortin family (cf. [6]).

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