Isolation of a novel calcium-binding protein from *Streptomyces erythreus*

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A small, acidic protein has been isolated from *Streptomyces erythreus*. Like eukaryotic calcium-binding proteins, it exhibits a calcium-dependent shift in mobility on SDS-polyacrylamide gel electrophoresis. Its N-terminal amino acid sequence contains a potential calcium-binding site which shows striking homology to the corresponding sites in calmodulin. Direct binding studies using ^{45}Ca showed that the protein, either purified or in cell-free extracts, is capable of binding calcium with high affinity.

Streptomyces erythreus *Calcium-binding protein* *Calmodulin*

1. INTRODUCTION

Many of the diverse intracellular biochemical effects of Ca^{2+} are mediated by a limited number of homologous calcium-binding proteins, among which calmodulin is prominent for its versatility and wide distribution in eukaryotic cells [1,2]. No protein resembling calmodulin has yet been characterized from prokaryotic cells [3–5]. However, acylcarrier protein from *Escherichia coli* appears to bind 2–3 mol Ca^{2+} per mol protein with low affinity, and an acidic, 22-kDa protein from *Salmonella typhimurium* is reported to be distinct from acylcarrier protein and to bind calcium [6]. Almost all well-established roles for calcium in bacteria involve either the plasma membrane [7], the cell surface [8] or the activation of exocellular enzymes [9]. An exception is the massive accumulation of Ca^{2+} that occurs during sporulation in both *Bacillus* [10] and *Streptomyces* [11]. We have now (serendipitously) isolated a protein from cell extracts of differentiating *Streptomyces erythreus* which bears a significant structural resemblance to authentic intracellular calcium-binding proteins.

2. MATERIALS AND METHODS

2.1. *Isolation of* S. erythreus *calcium-binding protein*

Growth of *S. erythreus* CA 340 and preparation of cell-free extracts were as previously described [12]. The calcium-binding protein was first noticed as a prominent side-product during the attempted purification of acylcarrier protein from *S. erythreus* using methods routinely successful with *E. coli* [13,14]. It was purified to homogeneity by the following method: solid ammonium sulphate (476 g/l) was added to the extract, at 4°C, and the precipitated proteins removed by centrifugation and discarded. Additional solid $(NH_4)_2SO_4$ (101 g/l) was added to the supernatant, the mixture was brought to pH 3.9 using acetic acid, and left overnight. The pelleted proteins were dialysed against 10 mM Pipes–KOH buffer, pH 6.2, and applied to a column of DEAE–cellulose. The target protein was eluted using a gradient of 0–0.5 M LiCl, emerging at approx. 0.3 M LiCl. On average, 30 mg homogeneous protein was obtained from 400 g cell paste.

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2.2. *Amino acid sequence analysis*

The N-terminal sequence of a sample of purified, salt-free *S. erythreus* protein was determined by automatic Edman degradation in an updated Beckman 890B spinning cup sequenator. Phenylthiohydantoin (PTH) amino acids were identified by HPLC [15].

2.3. *SDS-gel electrophoresis*

SDS-gel electrophoresis was carried out in slab gels (17.5 or 20% acrylamide) by the method of Laemmli [16] as previously described [12].

2.4. *Detection of calcium-binding proteins by direct binding of ^{45}Ca*

After separation by SDS-polyacrylamide gel electrophoresis, proteins were electrophoretically transferred to a nitrocellulose membrane as in [17]. Transfer was performed in a BioRad Transblot apparatus at 4°C. Detection of calcium binding was carried out as in [18].

3. RESULTS

3.1. *Preliminary characterisation of the putative calcium-binding protein*

The amino acid composition of the *S. erythreus* protein (to be published) shows, as expected, a preponderance of potentially acidic residues, but the composition is different from that of known calcium-binding proteins. The observed mobility of the protein during SDS-polyacrylamide gel electrophoresis corresponds to an apparent subunit M_r of 15400 in the presence of Ca^{2+}, and 15800 in the presence of added EGTA (fig.1). Calmodulin also shows a decrease in mobility in the presence of EGTA [19], but it is more pronounced (fig.1). Gel filtration of the *S. erythreus* protein on a column of Sephadex G-75 calibrated with proteins of known relative molecular mass, indicated a native M_r of 21000 (unpublished). It is therefore likely that the protein, as isolated, exists as a monomer.

3.2. *N-terminal amino acid sequence*

The sequence of the first 40 amino acid residues from the N-terminus of the *S. erythreus* protein is presented in fig.2a. The structural homology between one portion of this sequence and the sequence in each of the 4 calcium-binding domains of bovine brain calmodulin [20] is striking (fig.2b).

Fig.1. 17.5% SDS-polyacrylamide gel electrophoresis of *S. erythreus* protein and calmodulin. Proteins were stained with Coomassie brilliant blue. (a) Calmodulin + 1 mM Ca^{2+}, (b) calmodulin + 10 mM EGTA, (c) *S. erythreus* protein + 1 mM Ca^{2+}, (d) *S. erythreus* protein + 10 mM EGTA, (e) M_r standards: bovine serum albumin (M_r 68000), ovalbumin (M_r 43000), carbonic anhydrase (M_r 31000) and lysozyme (M_r 14300).

Secondary structure prediction using the Chou-Fasman method [21] shows that residues 1–10 and 18–35 are probably arranged in α-helices and the intervening region is predicted to be β-turn. This arrangement, in which α-helices flank a β-loop, is characteristic of all known 'EF-hand' calcium-modulated proteins [22].

3.3. *Calcium-binding properties of* S. erythreus *protein*

After SDS-polyacrylamide gel electrophoresis and electrophoretic transfer of the separated pro-

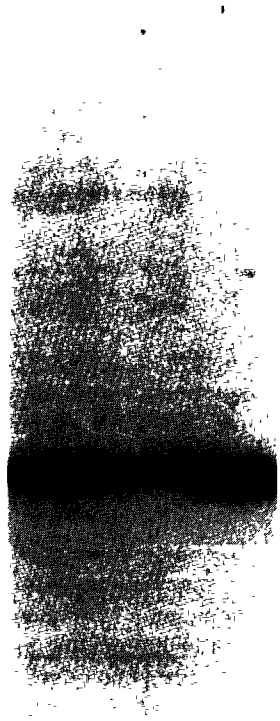
(a)

5 10 15 20 25 30 35 40

ADRLKKRFDRWDFDGNGALE RADFEKEAQHIAEAFGKDAG

(b)

			* * * * * *
S erythreus	protein		D F D G N G A L E R A D F
Calmodulin	domain	I	D K D G D G T I T T K E L
		II	D A D G N G T I D F P E F
		III	D K D G N G Y I S A A E L
		IV	N I D G D G Q V N Y E E F



a b c

Fig.3. Autoradiograph showing ^{45}Ca binding to *S. erythreus* protein; proteins were separated by SDS–PAGE on a 20% gel and transferred to a nitrocellulose membrane. The membrane was incubated for 10 min with 10 mM imidazole–HCl buffer, pH 6.8, containing 60 mM KCl, 5 mM $MgCl_2$ and 1 μCi/ml $^{45}CaCl_2$, washed twice with 50% ethanol, and autoradiographed for 48 h; (a) *S. erythreus* cell-free extracts (200 μg total protein); (b) as (a) but 75 μg protein; (c) purified *S. erythreus* protein (5 μg).

Fig.2. (a) N-terminal amino acid sequence of *S. erythreus* protein. The potential calcium-binding site is underlined. (b) Sequence comparison between the *S. erythreus* protein and the calcium-binding sites of bovine brain calmodulin. The asterisks denote the positions likely to provide ligands for Ca^{2+}.

teins to nitrocellulose membrane, high-affinity calcium-binding proteins can be recognized by their ability to take up ^{45}Ca from solution and retain it during subsequent washing [18]. In initial experiments using short transfer times (1 h) we found that purified *S. erythreus* protein was revealed by autoradiography of the nitrocellulose membrane after exposure to ^{45}Ca and therefore appeared to bind calcium (fig.3). Presumably, sufficient native structure is retained, or regained after transfer, to allow the calcium-binding site to function under these conditions. When the experiment was repeated using cell-free extracts from differentiating *S. erythreus*, the electrophoretic transfer was nearly complete after 24 h, as judged by subsequent staining of the original gel for protein. However, only one major protein species was radiolabelled by incubation of the nitrocellulose membrane with ^{45}Ca-containing buffer, and this had the same apparent M_r as the purified calcium-binding protein from *S. erythreus* (fig.3).

4. DISCUSSION

Our results show that extracts of differentiating *S. erythreus* apparently contain a single protein species, of approximately the same M_r as eukaryotic calmodulins, which can bind calcium with high affinity under certain conditions. Part of its N-terminal amino acid sequence shows significant homology with the calcium-binding domains of calmodulin. It is interesting to compare this protein with Protein S, the myxospore coat protein isolated from the Gram-negative bacterium *Myxococcus xanthus* [8]. Protein S contains 4 internally repeated domains, each with weak homology to calmodulin, and the presence of Ca^{2+} (10^{-3} M) is required to promote its association with the outer surface of the myxospore [23]. It is not known yet whether the *S. erythreus* protein contains internally repeated domains, nor whether it is developmen-

tally regulated in the way Protein S seems to be [8]. At present, the *S. erythreus* protein seems to be distinct from both calmodulins and Protein S. It does not activate 3′5′-cAMP phosphodiesterase (C.M. Lowry, unpublished), nor does it cross-react with specific antibodies raised against bovine brain calmodulin (M. Bardsley, unpublished). At the same time, its high affinity for Ca^{2+} seems more typical of intracellular calcium-binding proteins. Further work will be required to clarify both the structure and the biological role of this protein.

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