ON THE CALCIUM-BINDING ABILITY OF THE SYNTHETIC EVOLUTIONARY ANCESTOR OF CALCIUM-BINDING PROTEINS

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1. Introduction

A number of calcium-binding proteins have a domain structure and the domain primary structures are rather similar. Parvalbumins have three domains, two of them are able to bind calcium [1]. Homology of this kind can be found in troponin C [2] and myosin alkali light chains [3] having four domains.

Similarity of the primary and spatial structures of calcium-binding proteins and their domains suggested that there was a common evolutionary ancestor of these proteins and that subsequently gene duplication took place [4]. The ancestor structure was predicted [5] to be a 40-membered peptide with the following amino acid sequence:

H – Glu – Gln – Thr – Asp – Asp – Glu – Ile –
Lys – Glu – Val – Leu – Lys – Ala – Phe – Asp –
Lys – Asp – Gly – Gly – Gly – Arg – Ile – Asp –
Phe – Glu – Glu – Phe – Val – Lys – Leu – Ile –
Leu – Gly – Val – Thr – Gly – Glu – Gly – Ala –
Arg – OH

A calcium-binding peptide of this primary structure is unknown in nature at present, and isolation of the calcium-binding domain structurally corresponding to the ancestor failed; the isolated domain of carp parvalbumin was unable to bind calcium [6,8]. Chemical synthesis of the ancestor peptide chain would be a direct verification of the hypothesis. We have synthesized this peptide and have found that it possesses a specific calcium-binding activity.

2. Materials and methods

Pentafluorophenyl esters of amino acids were synthesized according to published methods [7]. Amino acid derivatives (Reanal, Hungary) were used for the synthesis. Sephadex (Pharmacia, Sweden) was used for purification of synthesized peptides. Purification of blocked peptides was carried out on Sephadex LH-20 in dimethylformamide. Sephadex G-25 in 0.02 M NH_4HCO_3, pH 8.0, and DEAE–Sephadex A-25 in 0.02 M NH_4HCO_3, pH 8.0 (KCl gradient from 0.0–1.0 M) were used for isolation of the unblocked final tetracontapeptide. Amino acid analysis was made with a Durrum D-500 analyzer. The amino acid sequence was determined with the automatic Beckman 890C sequencer. The N- and C-terminal amino acids were analyzed by standard methods using dansyl chloride and carboxypeptidases A and B, respectively. The calcium-binding ability was determined as in [8]. $^{45}CaCl_2$ (17 mCi/mg) was from the Radiochemical Centre Amersham (England).

3. Results and discussion

We synthesized the peptide of the above mentioned structure using pentafluorophenyl esters of *tert*-butyloxycarbonyl amino acids. Synthesis was

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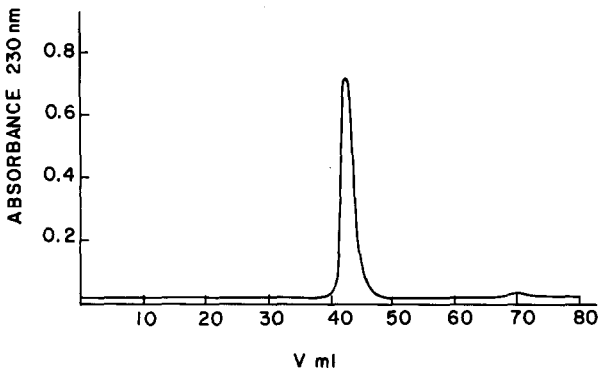

Fig.1. Analytical gel chromatography of the synthetic peptide. Column 1 × 90 cm with Sephadex G-50f. Buffer: 50 mM Tris–HCl, pH 7.2 + 30 mM KCl; the rate was 10 ml/h.

carried out in solution according to the general scheme 7 + {7 + [(5+7) + (7+7)]}. The fragment 15–26 was synthesized in collaboration with Professor H.-D. Jakubke (Martin-Luther-Universität, Halle, DDR).

A detailed description of the tetracontapeptide synthesis will be published later. After removing masking groups with HF/anisole the resulting peptide was purified by gel filtration and ion exchange chromatography. The analytical gel filtration showed that the product was homogeneous (fig.1). The synthesized tetracontapeptide had Glu as a N-terminal and Arg as a C-terminal amino acid, and the following amino acid composition (the calculated values are given in parentheses):

Asp 5.13 (5), Thr 1.73* (2), Glu 6.71 (7), Gly 6.15 (6), Ala 2.1 (2), Val 3.0 (3), Ile 3.0 (3), Leu 3.15 (3), Phe 3.0 (3), Lys 3.92 (4), Arg 1.8 (2).

Determination of the amino acid sequence of the synthesized peptide gave the following sequence for the N-terminal part of the peptide chain:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad 5 \\ \mathrm{H-Glu-(Gln)-Thr-Asp-Asp-Glu-Ile-} \\ \qquad\qquad\quad 10 \qquad\qquad\qquad\qquad\qquad\quad 15 \\ \mathrm{Lys-Glu-Val-Leu-Lys-Ala-Phe-Asp} \end{array}$$

which coincides with the predetermined sequence.

* Losses on hydrolysis (24 h, 6 N HCl, 110°C) were not taken into account

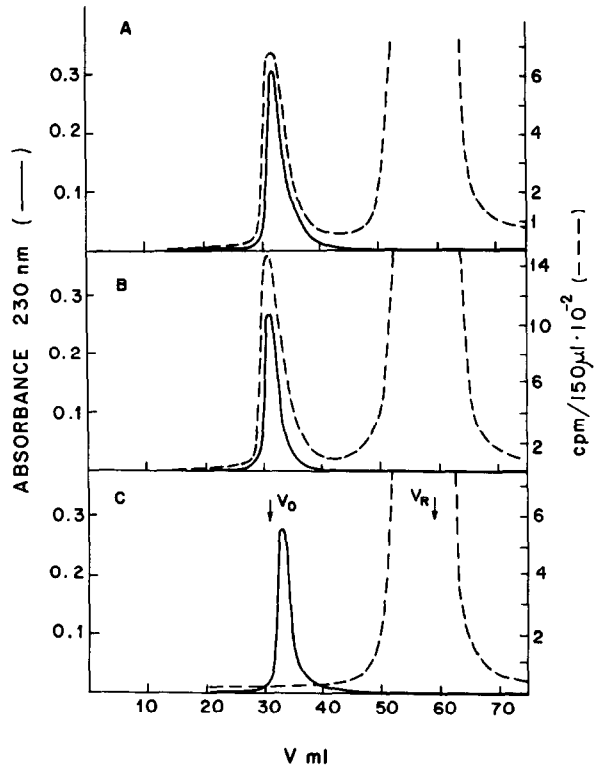

Fig.2. Calcium-binding ability of the synthetic peptide (A), that of carp parvalbumin (B) and that of fragment 76–108 of carp parvalbumin (C). After incubation for 30 min with 1 mCi $^{45}CaCl_2$ in 0.2 ml 0.05 M Tris–HCl buffer containing 30 mM KCl the substance tested was loaded onto the column of Sephadex G-25f and eluted at the rate of 80 ml/h. Fractions of 2.5 ml were collected.

The result of determination of the calcium-binding ability is given in fig.2. The chromatograms show that the synthesized peptide (in contrast to parvalbumin fragment 76–108 [6]) possesses a specific calcium-binding activity similar to that of parvalbumin. The ratio of the bound calcium to the quantity of the tested peptide in the peak (as in [8]) indicated that the sample contained not more than 10% of the active peptide.

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