

THE AMINO ACID SEQUENCE OF THE PIKE (*ESOX LUCIUS*) PARVALBUMIN III

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1. Introduction

The primary structure of major parvalbumins of hake (*Merluccius merluccius*) [1, 2] and of carp (*Cyprinus carpio*) [3] have been determined recently. The comparison of the two sequences has confirmed the homology of these proteins typical of aquatic cold blooded vertebrates [4, 5].

We report here the amino acid sequence of the major component of the two parvalbumins extracted from pike muscle. This protein has, by contrast with the rather conservative amino acid compositions of the other parvalbumins, and unusual composition characterized by a high content of 17 lysine residues and by the absence of cysteine, proline, tyrosine and tryptophan. The molecule has an isoelectric point of 5.0 and strongly binds two calcium ions [6, 7].

2. Materials and methods

The protein was prepared and characterized as described previously [6, 7].

Tryptic digestion was performed at 37°C in 0.2 M NH_4HCO_3 brought to pH 7 with formic acid by addition of 2% (w/w) trypsin [8]. The procedure, lasting 8 hr, was repeated twice in order to ensure complete digestion of the native protein.

The chymotryptic digestion was carried out in 0.2 M phosphate buffer, pH 8.5 with 1% (w/w) chymotrypsin for 15 hr at 37°C.

CNBr fragmentation was performed as described earlier [9]. The resulting fragments were isolated by gradient elution chromatography on DEAE Sephadex A-25.

The isolation of the individual peptides produced by enzymatic digestions was according to the following sequence of operations: gel filtration on a Biogel P₄ column (2.5 × 100 cm) equilibrated in 0.05 M NH_4HCO_3 , further resolution of the fractions by chromatography on polystyrene sulfonic resins (Dowex 50 × 2, Technicon Chromobeads P) in pyridine-acetic acid buffers and on DEAE cellulose (Whatman DE 32), with final purification by chromatography or electrophoresis on cellulose thin layer (1 mm) and on paper.

The N-terminal acetylated chymotryptic peptide was obtained from a 2 hr, 2% (w/w) chymotryptic digest of the succinylated protein [10] by passing the acidified mixture through a Dowex 50 × 2 column (0.9 × 15 cm), H⁺ form. The peptide was recovered by elution with water.

The amino acid analyses of the peptides, after hydrolysis in 6 N HCl for 24 hr, were made with a modified Beckman amino acid analyzer 120B using the two column system [11].

The amino acid sequences of the peptides were elucidated using digestions with carboxypeptidase A (EC 3.4.2.1), carboxypeptidase B (EC 3.4.2.2), leucine amino peptidase (EC 3.4.1.1) and the Edman-dansyl method [12]. Dansyl amino acids were identified on (7.5 × 7.5 cm) polyamide layers (Cheng Chin Trading) [13].

The presence of the N-terminal acetyl-alanine residue and the amino acid sequences of the tryptic octapeptides T₃ and T₂₀ have been confirmed by mass spectrometric analysis of the permethylated materials.

The position of the amide groups have been assigned on the basis of the electrophoretic mobilities of the peptides on paper at pH 6.5 [14] and in ambiguous

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