PARAMYOSIN: CHEMICAL EVIDENCE FOR CHAIN HETEROGENEITY

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1. Introduction

Paramyosin is a major constituent of the smooth adductor muscles of many molluscs, where it forms the backbone of the thick (myosin containing) filaments [1]. It is a rod-shaped molecule about 130 nm long [1] and is constructed from two α-helical chains tightly bound into a coiled-coil configuration [2,3]. The chemical nature of these chains has not been investigated in any great detail [4] and it is not known whether the population is homogeneous or not.

In this study we examine the cysteine containing peptides of scallop (*Pecten maximus*) paramyosin and establish that the population of chains in this species is heterogeneous.

2. Materials and methods

2.1. *Paramyosin*

Crude paramyosin was prepared from the opaque adductor of the scallop *P. maximus* by the method of Johnson et al. [5]. It was purified by four-fold recrystallisation [6] and the last traces of contaminating proteins were removed by dissolving in 0.6 M KCl, 10 mM K phosphate pH 6.2 and dialysing against 25 mM K pyrophosphate pH 7.0 at 4°C when only the paramyosin precipitated. This paramyosin was then dialysed exhaustively against 5 mM HCl and stored frozen at −20°C until required. This material produced a single band when examined by SDS gel electrophoresis at loadings in excess of 0.1 mg on 7% polyacrylamide slabs in a 0.1% SDS, 0.1 M Tris-bicine, pH 8.9 running buffer.

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2.2. *Carboxymethylation*

Paramyosin samples were freeze dried and dissolved in 6 M guanidine hydrochloride, 0.1 M Tris–HCl pH 8.0 to a concentration of approximately 10 mg ml^{-1}. A ten-fold excess of dithiothreitol was then added and the reaction continued for 1 h at room temperature. A two-fold excess (over total thiol) of iodo-[^{14}C]-acetic acid was then added and the reaction continued for 1 h in the dark. The reaction was then terminated by the addition of an approximately 100-fold excess of dithiothreitol. Radioactive by-products were then removed from the labelled protein by gel filtration on Sephadex G-50 in 6 M guanidine hydrochloride, 0.1 M Tris–HCl pH 8.0. The protein was then dialysed against 5 mM HCl and freeze dried.

2.3. *Digestion*

S-carboxymethyl-paramyosin (10 mg/ml) was digested with termolysin (0.5 mg/ml) in N-ethyl morpholine acetate pH 8.3 for 8 h at 45°C.

2.4. *Peptide purification*

Thermolytic peptides were separated by cation exchange chromatography on sulphonated polystyrene beads (Locarte resin L15) using a gradient of pyridinium acetate (Walker et al. unpublished). The major radioactive peptides were subsequently purified by paper electrophoresis at pH 6.5 and at pH 3.5. Amino acid analyses of purified peptides were carried out on a Durrum D-500 amino acid

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analyser and sequences were determined by the dansyl-Edman procedure.

2.5. *Analytical techniques*

Protein concentration was determined by the Lowry procedure taking triplicates in each case. Radioactivity was measured by dissolving 0.02--0.2 ml samples in Brays scintillation fluid and counting on a Nuclear Chicago Isocap/300 liquid scintillation counter. All determinations were carried out in duplicate. The mol. wt of paramyosin was taken as 205 000 [2].

3. Results

The cysteine content of *P. maximus* paramyosin was established as 2.06 residues per chain (3 determinations) on the basis of incorporation of radioactive iodoacetic acid. This compares well with the value obtained for *Mercenaria* paramyosin which also indicated 2 cysteine residues per chain [6].

When submitted to paper electrophoresis and auto-radiography, the thermolytic digests of *P. maximus* paramyosin labelled with iodo[^{14}C]-acetic acid showed 3 major radioactive peptides. To determine the relative amounts of these peptides

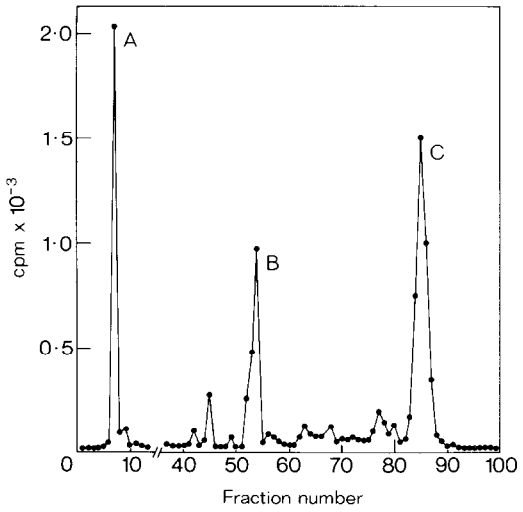

Fig.1. Separation of thermolytic peptides of paramyosin by cation exchange chromatography. For details see section 2.3. Note the presence of three thiol peptides.

Table 1

Cysteine Peptide Sequences from *P. maximus* Paramyosin

Peptide A	Leu–Glx–Asx–Ala–Cys–Asx–Thr
Peptide B	Phe–Glx–Lys–Glx–Cys
Peptide C	Leu–Cys–Arg–Arg

present and to prepare samples for sequence studies these peptides were fractionated by cation exchange chromatography when three major peaks were obtained (see fig.1). The three peptides, A, B and C were present in the ratio 1.0 : 0.81 : 2.1 and were radiochemically pure as assessed by paper electrophoresis at pH 6.5 and pH 3.5. The sequences of these peptides were determined and are shown in table 1.

4. Discussion

If all paramyosin chains were identical only two unique sequences containing cysteine would occur in the thermolytic digest. The existence of three peptides containing cysteine is therefore compelling evidence for chain heterogeneity. Although the full extent of heterogeneity cannot be assessed by the present approach, the stoichiometry in which peptides A, B and C were isolated is compatible with only two chains. As peptide C is present is twice the amount of either peptides A or B, this is clearly the peptide common to both chains, while the fact that peptides A and B present in approximately equal amounts is suggestive of the presence of $\alpha\beta$ dimers, although a population of homodimers cannot be ruled out.

The occurrence of strikingly similar amounts of each chain in mollusc muscle suggests that both genes are expressed in a coordinate fashion in all muscle cells. The demonstration of native $\alpha\beta$ dimers of paramyosin would confirm this. It is interesting to note that chain heterogeneity has also been demonstrated in the closely related α-protein, tropomyosin [7,8].

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