A COMPARISON OF FIBROBLAST AND SMOOTH MUSCLE MYOSINS

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1. Introduction

Myosin is a major component of muscle and plays a well understood role in the contraction mechanism. It is a large protein of about 500 000 daltons and is made up of two heavy chains and several light chains. Different kinds of muscle – fast and slow skeletal, smooth and cardiac – have been shown to differ in both their heavy and light chain components [1–4]. Many non-muscle tissues have been shown to contain a protein similar to myosin, which is presumed to function in cellular motility (reviewed in refs. 5 and 6). These cytoplasmic myosins have been well characterised from some sources, for example from platelets [7], and fibroblasts [8] and appear to be similar to muscle myosins by several criteria – in size, enzymatic activity and ability to bind to actin.

Structural comparisons between cytoplasmic myosin and the various muscle myosins are of great interest and have not yet been made. In this paper I examine the possible similarity between fibroblast and smooth muscle myosins and have shown that the two myosins have identical electrophoretic patterns of heavy and light chains on SDS gels. Comparison of their co-migrating light chains by tryptic fingerprinting has indicated a close similarity of ^{35}S-methionine peptides from fibroblasts with unlabelled peptides from smooth muscle. Comparison of the heavy chains, however, by limited proteolysis of the native myosins has shown that, while similar, the myosin heavy chains from fibroblast and smooth muscle are not identical.

2. Experimental

Fibroblasts from the skin of 11-day chick embryos which had been dissociated with trypsin were grown in Eagle's minimal essential medium (MEM) + 10% horse serum, in 9 cm Sterilin Petri dishes. At confluence the cells were transferred to fresh dishes at about 10^6 cells/dish with MEM containing $^1/_{10}{}^{th}$ the normal methionine and supplemented with ^{35}S-methionine (Amersham Radiochemical Centre) at · 1 mCi/dish. The cells were grown for 3 days and then harvested by scraping off the dishes. The fibroblast myosin was purified by a modification of the method of Adelstein et al. [8] using a small amount of 12-day embryo chick brain as a non-muscle carrier as described elsewhere [9].

Smooth muscle trypsin was prepared from chicken gizzards by the method of J. Kendrick-Jones [4] followed by the additional step of gel filtration through Sepharose 4B.

The fibroblast myosin light chains were purified and extracted from a preparative SDS slab gel [9].

Partially purified smooth muscle (chicken gizzard) myosin light chains were a generous gift of Dr. J. Kendrick-Jones. The 20 000 dalton gizzard light chain, G1, contained a trace of the 17 000 dalton light chain, G2. In turn the purified G2 contained approximately 25% of G1. The corresponding radioactive fibroblast and unlabelled smooth muscle light chains were mixed, oxidised with performic acid and digested with $^1/_{50}{}^{th}$ of their weight of trypsin (Worthington purest) overnight. The tryptic digests were electrophoresed on Whatman No. 1, first at pH 6.5 and then in the 2nd dimension at pH 2.1. The smooth muscle light chain peptides were detected by reacting with fluorescamine [10] and the fibroblast methionine peptides by autoradiography.

For limited proteolytic digestion the native myosins

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in a high salt buffer (0.6 M NaCl, 0.015 M Tris–HCl pH 7.4, 0.1% β-mercaptoethanol) were mixed with either trypsin, chymotrypsin, papain, (from Sigma) or pronase (BDH) at $^{1}/_{200}{}^{th}$ the weight of the myosin, except for chymotrypsin at $^{1}/_{100}{}^{th}$. The digestion was allowed to proceed at 37°C for 30 min and stopped by boiling in the SDS-sample buffer for the analytical gel.

The SDS gel electrophoresis was performed as in [9] or for fig. 3 as described by Laemmli [11].

3. Results and discussion

3.1. *A Comparison of fibroblast and smooth muscle myosins on SDS gels*

A 10% gel and its autoradiograph is shown (fig. 1) to compare the myoson from ^{35}S-methionine-labelled fibroblasts with unlabelled smooth muscle myosin. The autoradiograph of the gel indicated co-migration of both the heavy and light chains of fibroblast myosin with the respective components of smooth muscle myosin. On gels loaded lightly the heavy chains of smooth muscle myosin (though not skeletal or cardiac) are seen to be a doublet [4]. This is also seen with fibroblast myosin, though the heavy loading of the gel in fig. 1 that is required to show the light chains has obscured the 'doublet' in both the gel and autoradiograph.

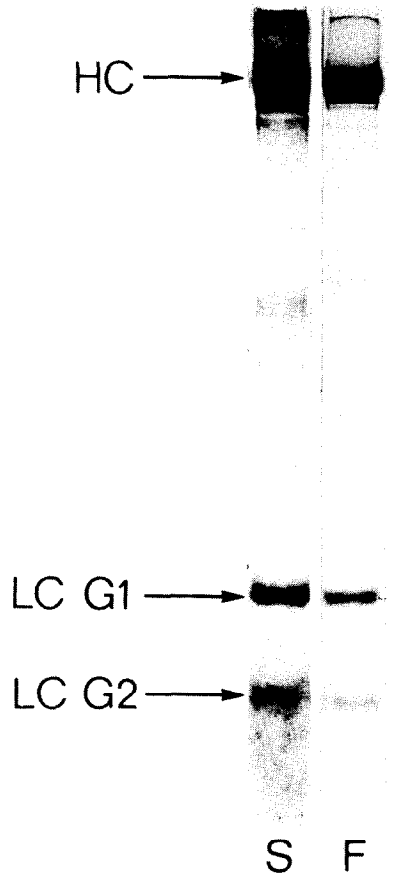

Fig. 1. A comparison of smooth muscle and fibroblast myosins by SDS gel electrophoresis. The smooth muscle myosin (12 μg of protein) and fibroblast myosin (20 000 cpm) were mixed and run on a 10% slab gel, as described in the Experimental section. S = smooth muscle myosin, a photograph of a stained, dried down section of a gel. F = fibroblast myosin, the autoradiograph of the same section. HC = heavy chain, LC G1 = the 20 000 dalton light chain and LC G2 = the 17 000 dalton light chain.

3.2. *A comparison of the tryptic peptides of fibroblast and smooth muscle myosin light chains*

The partially purified smooth muscle light chains were mixed with their respective ^{35}S-methionine-labelled fibroblast light chains which had been extracted from a preparative SDS gel. Together they were oxidised and digested with trypsin and the fingerprints are shown in fig. 2. The unlabelled fingerprints and their respective autoradiographs consisted of a number of strongly stained or radioactive areas areas and a number of strongly stained or radioactive areas and a number of minor ones, probably arising from secondary cleavages, deamidation, etc. Both stronger and weaker radioactive peptides from fibroblast light chains overlapped the fluorescamine stained peptides from the smooth muscle light chains. It should be noticed that in the G2 map two areas are marked with arrows for which fluorescamine staining extended beyond the radioactive areas, possibly suggesting that the fluorescamine staining represented two peptides, only one of which was radioactive. In all other cases the contour of the radioactive peptides corresponded closely to that of the stained peptides. The number of major radioactive peptides is of the order expected from the amino acid analysis of smooth muscle light chains [4]. In the G1 map fluorescamine staining and radioactive material (in an amount equal to about two methionines) remained at the origin and may represent

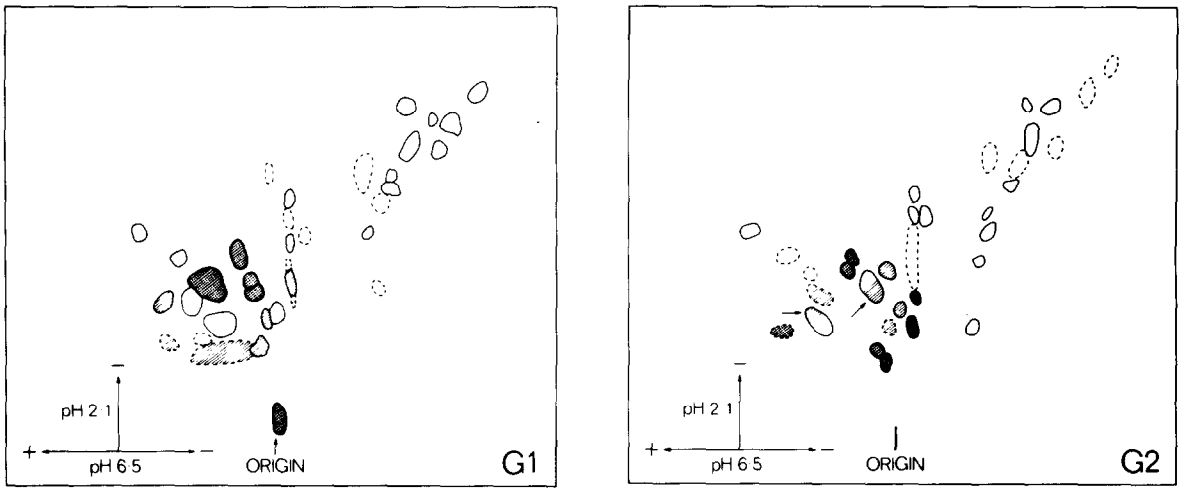

Fig. 2. Tryptic fingerprints of the smooth muscle and ^{35}S-methionine-labelled fibroblast light chains. G1 = the 20 000 dalton light chain, G2 = 17 000 dalton light chain. The peptides that reacted strongly with fluorescamine are outlined with a solid line, those reacting weakly with fluorescamine with a broken line. The strongly radioactive peptides are marked with cross-hatching in both directions; the weakly radioactive peptides with cross-hatching in one direction. In the G2 map the arrows indicate 2 peptides for which the fluorescamine staining area extended further than the radioactive area, possibly suggesting that the fluorescamine staining represented two peptides, only one of which was radioactive. In all other cases the radioactive peptides corresponded closely to the stained peptides.

insoluble peptides or material that has only been partially digested.

The close overlapping of the labelled methionine peptides from fibroblast light chains with peptides from smooth muscle light chains suggests a close similarity between them. Identity, however, can only be established by a complete sequence analysis.

Recent work on artificially stimulated [12] and on cross-reinnervated muscle [13] has shown that the pattern of light chains relates to the activity of the muscle. If then the light chains from fibroblasts and smooth muscle are shown to be the same this would not only have interesting genetic implications, but would also suggest a closer functional similarity between the two myosins than has previously been considered.

3.3. *A comparison of fibroblast and smooth muscle myosin heavy chains*

Because of the high molecular weight of the myosin heavy chains and the very large number of tryptic peptides that are formed, an alternative approach for comparing the heavy chains was used. Limited digestion of the native myosins from skeletal, cardiac and smooth muscles with different proteolytic enzymes has been shown to give distinctive fragments of different molecular weights when analysed by SDS gel electrophoresis [9]. The pattern of fragments of smooth muscle and fibroblast myosin due to digestion with 4 enzymes is shown in fig. 3. The pattern of cleavage of the same myosin by different enzymes is noticeably different for each enzyme. Comparing the autoradiographs with the stained gels and considering both the position and intensity of the bands, it can be seen that the degree of similarity of the proteolytic fragments from the two myosins varies according to which enzyme is used. Thus, for example, papain gives a very similar pattern of fragments whereas for chymotrypsin the fragments differ considerably. Since the digestion was performed on the native myosins, the fragments produced, while suggestive of differences in primary sequence, may have arisen as a result of secondary modification of the myosins, such as methylation, which may change the sites of cleavage by altering the tertiary structure. Nevertheless the result indicates that differences in the myosin heavy chains do exist, though whether in their primary sequence or their tertiary structure it is not yet possible to say.

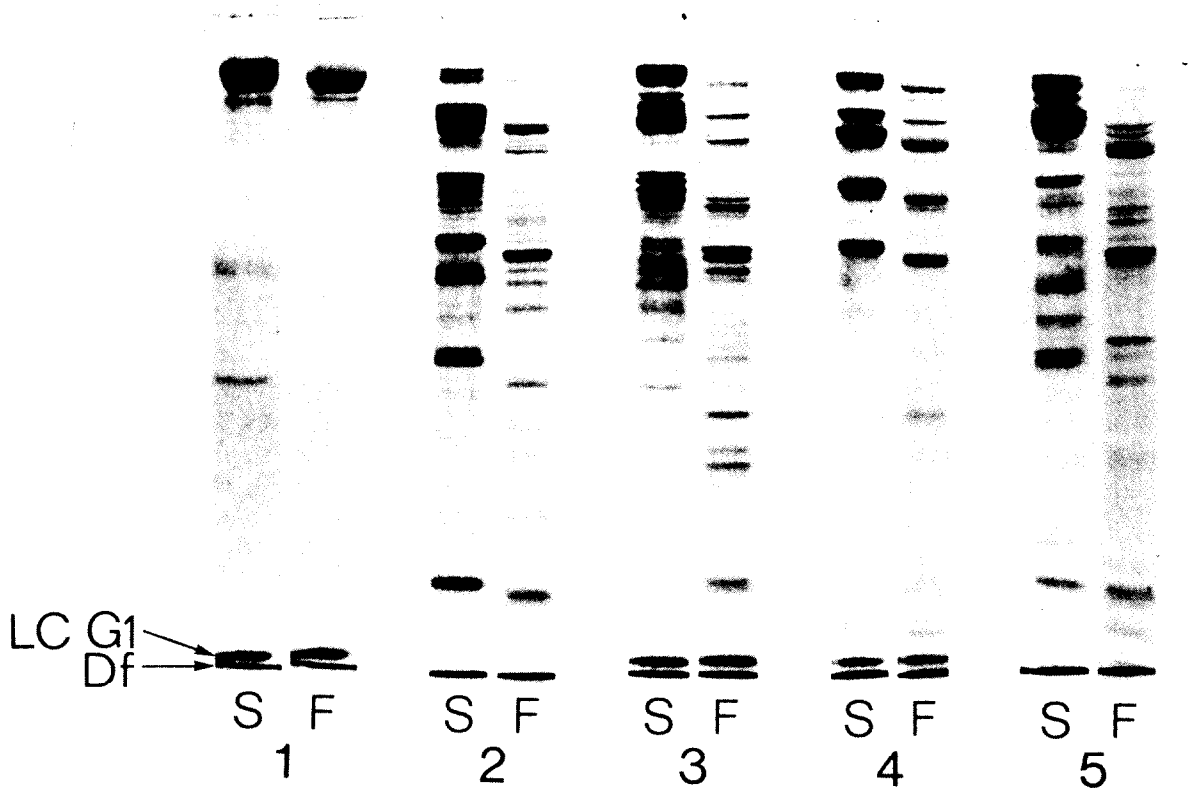

Fig. 3. A comparison of fragments formed by limited proteolytic digestion of smooth muscle and fibroblast myosins. The native smooth muscle myosin (12 μg of protein) and fibroblast myosin (10 000 cpm) were mixed and digested for 30 min with either trypsin, chymotrypsin, papain or pronase and run on a 10% SDS slab gel as described in the Experimental section. The gel was run from top to bottom. 1 = undigested myosin, 2 = trypsin, 3 = chymotrypsin, 4 = papain, 5 = pronase. S = smooth muscle myosin, photographs of stained, dried down sections of a slab gel. F = fibroblast myosin, autoradiographs of the corresponding sections. The dye front is marked Df. In some cases the undigested 20 000 dalton light chain is visible (LC G1).

It will be of interest now to examine other non-muscle myosins by these methods and to determine whether they all belong to a single type.

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