Chicken Muscle and Fibroblast Actin Structure

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(Received 26 July 1976)

Double labelling and the isolation of peptides specific to muscle actin indicates that completely homologous 20-residue peptides can be produced from the C-terminal regions of muscle and chicken-embryo fibroblast actins by treatment with CNBr. By quantification of the amount of this peptide that can be produced from acetone-dried powders by CNBr treatment, 6.8% of the protein of the fibroblasts has been estimated to be actin.

A protein with properties similar to the contractile muscle protein actin has been found in a wide variety of cells (Pollard & Weihing, 1974). Studies indicate that actins from muscle and non-muscle sources have similar but not identical amino acid sequences. Chicken brain actin contains six tryptic peptides not found in chicken muscle actin and lacks one of the tryptic peptides of muscle actin (Greunstein & Rich, 1975). The actins from 3T3 and HeLa cells are also very similar to muscle actin in structure, but are not identical with it (Greunstein *et al.*, 1975). The nature and location of the structural alterations in the above cases have not been identified, but a specific amino substitution of the valine-129 residue of muscle actins by a threonine residue has been shown to occur in the actin of human blood platelets (Elzinga *et al.*, 1976). This finding demonstrates that muscle and non-muscle actins are products of separate genes.

Whether non-muscle actin has a function and mechanism similar to those of muscle actin is not known (Pollard & Weihing, 1974). Information concerning the structure and amount of actin in non-muscle cells may be expected to be of value in determining its role. The degree of homology between muscle and non-muscle actins will depend on the amount of actin structure that is essential to its function and on whether muscle and non-muscle actins function identically. Examination of the structure of a variety of muscle actins has indicated that the amino acid sequence of this protein is very highly conserved (Elzinga & Collins, 1973). This suggests that a high degree of structural integrity is necessary for actin function. A knowledge of the degree of homology between muscle and non-muscle actins clearly will be of great value in determining if this is so and if muscle and non-muscle actins function identically. In addition, a knowledge of the amount of actin present in non-muscle cells may be an indication of the functional role of this protein. In muscle, large amounts of actin are required to form the thin filaments (Hanson & Huxley, 1957).

Since actin has no known catalytic activity, it would be expected that relatively large amounts of the protein would be required in non-muscle cells if it functions in these cells in a manner similar to its mechanism of action in muscle.

A method has been developed which allows the quantification of individual proteins in cells and tissues by the isolation of peptides unique to a given protein (Anderson, 1976). The use of this method to examine in non-muscle cells the amount of a variety of peptides found in muscle actin might be expected to provide information concerning the amount of actin present in these cells and the degree of homology between this actin and muscle actin. Such a procedure would be expected to be of particular value in examining the actin of chicken fibroblasts. Although approx. 8% of the protein of these cells has the same mobility on sodium dodecyl sulphate-containing gels as muscle actin (Bray & Thomas, 1975), only 0.2% of the protein of chicken-embryo fibroblasts has been recovered as purified actin (Yang & Perdue, 1972). This low yield makes difficult the examination of the degree of homology of this protein to muscle actin by the application of the normal methods of amino acid sequence determination. The isolation of individual peptides from the protein of the cells eliminates problems which could be encountered in protein purification, but still allows quantification of the protein and examination of the degree of homology.

In a previous study the amount of an 18-residue peptide that can be generated from the N-terminal region of muscle actin (carboxymethylated with iodoacetic acid) by digestion with trypsin was determined in fibroblasts (Anderson, 1976). Only very small amounts of this peptide can be generated from the carboxymethylated proteins of chicken-embryo fibroblasts. This finding indicates either that the amino acid sequences of muscle and fibroblast actins differ in this region of sequence or that only very small amounts of actin are present in the fibroblasts. To help distinguish between these possibilities, the

amount of two other peptides which can be generated from muscle actin has now been determined in fibroblasts.

Materials and Methods

The preparation of pure actin from chicken muscle and conditions for the growth of chicken-embryo fibroblasts and the preparation of acetone-dried powders from them have been previously described (Anderson, 1976). Chemical modifications of purified muscle actin with iodo[2-¹⁴C]acetic acid were also carried out as previously described (Anderson, 1976).

Preliminary experiments indicated that treatment of carboxymethylated chicken muscle actin with CNBr produces a 16-residue carboxymethylcysteine (CmCys)-containing peptide Lys-CmCys-Asp-Ile-Asp-Ile-Arg-Lys-Leu-Tyr-Ala-Asn-Asn-Val-Hsr (Hsr = homoserine), which is completely homologous to a peptide that can be generated from carboxymethylated rabbit muscle actin (Elzinga & Collins, 1973); this was designated 'peptide X2A'. A 20-residue CmCys-containing peptide completely homologous to a peptide generated from the C-terminal region of rabbit muscle actin by CNBr treatment (Elzinga & Collins, 1973) can also be generated from chicken muscle actin. This peptide has the structure Trp-Ile-Thr-Lys-Gln-Glu-Tyr-Asp-Glu-Ala-Gly-Pro-Ser-Ile-Val-His-Arg-Lys-CmCys-Phe and was designated 'peptide X2N'.

The amounts of these peptides in chicken-embryo fibroblasts were determined by treatment of mixtures of carboxy[¹⁴C]methylated purified chicken muscle actin and carboxy[³H]methylated acetone-dried powders of fibroblasts with CNBr and subsequent purification of the peptides X2A and X2N by methods previously described (Anderson, 1976). Standards were prepared by the addition of known amounts of purified actin to foetal calf serum proteins, which were then treated in the same way as the chicken-embryo-fibroblast acetone-dried powders. These standards allowed the calculation of amounts of actin sequence from the ³H/¹⁴C ratios obtained for peptides purified from mixtures. The ³H/¹⁴C ratios corrected for spillover were obtained by liquid-scintillation counting as previously described (Anderson, 1976).

Results and Discussion

Table 1 gives the ³H/¹⁴C ratios for peptides generated from mixtures of known amounts of iodo[¹⁴C]acetic acid-modified chicken muscle actin and known amounts of iodo[³H]acetic acid-modified proteins of chicken-embryo-fibroblast acetone-dried powders. To allow the calculation of peptide quantities from these ratios, carboxy[¹⁴C]methylated muscle actin was added to carboxy[³H]methylated foetal calf

Table 1. *Actin content of fibroblasts as measured by double labelling*

S₁, 0.75nmol, and S₂, 1.50nmol of purified actin from chicken muscle in 15mg of foetal calf serum protein. CEF₁, 12.0mg, and CEF₂, 26.3mg of protein from acetone-dried powders of chicken-embryo fibroblasts. Chicken-embryo-fibroblast cultures and acetone-dried powders from them were prepared as previously described (Anderson, 1976). The samples were carboxymethylated with iodo[³H]acetic acid (70mCi/mmol), and 100nmol of purified chicken muscle actin carboxymethylated with iodo[¹⁴C]acetic acid (1mCi/mmol) was added to each before peptide generation. ³H/¹⁴C ratios were determined by liquid-scintillation counting of portions of the purified peptides as previously described (Anderson, 1976).

Sample	Peptide	³ H/ ¹⁴ C ratio	Actin	
			(nmol)	(%, w/w)
S ₁	X2A	0.35:1	0.75	
	X2N	0.33:1	0.75	
S ₂	X2A	0.56:1	1.50	
	X2N	0.62:1	1.50	
CEF ₁	X2A	0.43:1	1.02	0.35
	X2N	8.16:1	19.43	6.80
CEF ₂	X2A	2.38:1	5.67	0.91
	X2N	17.72:1	42.19	6.73

serum protein to which known amounts of untreated purified muscle actin had been added before the treatment with iodo[³H]acetic acid. Generation and isolation of peptides from such mixtures showed that the ³H/¹⁴C ratios of the peptides so obtained was directly related to the amount of untreated actin added to the foetal calf serum proteins before carboxymethylation with iodo[³H]acetic acid. Thus the quantities of actin peptides given in Table 1 were calculated by using the ³H/¹⁴C ratios, and the ³H/¹⁴C ratios obtained when known amounts of actin were added. Since fibroblast actin appears to have the same molecular weight as muscle actin (Bray & Thomas, 1975), the amount of protein of mol.wt. 42000 necessary to give rise to the amount of peptide observed was calculated from these ratios. This allowed the estimates of actin content as a percentage of the protein given in Table 1.

It can be seen that only small amounts of peptide X2A can be generated from the proteins of the fibroblasts, whereas a relatively large amount of the peptide X2N is obtained. The most likely error in quantification by the method used would arise from the presence of contaminating peptides, which would give rise to an overestimate of the amount of peptide present. Therefore it is likely that the small amounts of peptide X2A found in the present study, and of the N-terminal tryptic peptide of muscle actin found in a previous study (Anderson, 1976), are due to a lack

of proteins with sequences identical with these peptides in the proteins of fibroblasts. Thus, if an actin-like protein is present in fibroblasts in relatively large amounts, it must differ in amino acid sequence from muscle actin in these regions. The generation of large amounts of peptide X2N from the protein of fibroblasts indicates that there is a large portion of protein in fibroblasts which can give rise to this peptide. Since the random occurrence of an amino acid sequence is 1 in 20^n , where n is the number of residues in the sequence, and peptide X2N contains 20 residues, it is very unlikely that any protein other than actin will contain the same amino acid sequence as gives rise to peptide X2N. The presence of peptides which co-purify with peptide X2N would lead to an overestimate of the amount of X2N. The isolated peptide was therefore treated with trypsin, and the dipeptide cysteinylphenylalanine was isolated from this digest by high-voltage paper electrophoresis and paper chromatography. It is unlikely that peptides contaminating peptide X2N would give rise to this peptide. Since no change in $^3\text{H}/^{14}\text{C}$ ratios was observed after this process, it appears that peptide X2N contains no contaminating peptides, a situation that would lead to an error in the estimation of the amount of peptide X2N present.

Thus, on the basis of the $^3\text{H}/^{14}\text{C}$ ratios of isolated peptides, and a mol.wt. of 42000 for fibroblast actin, it can be calculated that 6.8% of fibroblast protein is homologous to muscle actin. This is in good agreement with a previous estimate based on quantification of protein on polyacrylamide gels (Bray & Thomas, 1975).

The peptides X2A and X2N account for 10% of the sequence of muscle actin. Together with the N -terminal tryptic peptide examined in a previous study (Anderson, 1976), 15% of the sequence of muscle actin has now been looked for in fibroblasts. These studies indicated that 6.8% of the protein of chicken-embryo fibroblasts consists of a protein homologous to but not identical with muscle actin, and demonstrates in which regions of actin sequence the homologies and differences occur. Examination of peptides generated from other regions of the amino acid sequence of actin may be expected to provide more information that will allow a better understanding of fibroblast actin structure and function.

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