NON-IDENTITY OF MUSCLE AND NON-MUSCLE ACTINS

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SUMMARY: Tryptic peptide maps of actin prepared from chicken muscle and chick brain appear to be very similar, but not identical. Brain actin lacks at least one peptide found in muscle actin and its fingerprint contains approximately six additional peptides. Whether these differences between muscle and cytoplasmic actins are due to their synthesis from different genes or to post-translational modification is not yet known.

INTRODUCTION

Muscle actin has been subject to a high degree of evoluationary conservation. Peptide maps of a variety of mammalian and avian muscles are indistinguishable while those of frog, fish, and scallop differ in only 1, 2, and 8 peptides respectively (1). Recently, considerable interest has focused on the observation that non-muscle tissues also contain contractile proteins previously considered characteristic of muscle (see ref. 2 for a review of non-muscle contractile proteins). In particular, the actins isolated from non-muscle cells exhibit extensive similarities to muscle actin including similarities in molecular weight, polymerization-depolymerization properties, activation of myosin ATPase, and decoration by heavy meromyosin (2). Indeed, on the basis of these properties and the demonstration of indistinguishable tryptic peptide maps using [^{35}S]methionine labeled protein, Bray (3) has suggested that muscle and cytoplasmic actins of the same species are probably the same protein, i.e. probably the product of a single gene. Here we report a study of chick brain and muscle actin using [^{3}H]lysine and [^{3}H]arginine which should label almost all tryptic peptides. Using modifications of the

procedures described by Bray (3) and Fine and Bray (4), we have found apparent differences in the structures of chick muscle and chick brain actins as judged by differences in their peptide maps.

METHODS

Chicks were killed during or immediately after hatching by decapitation and the brains and thigh muscles removed and placed in ice cold Krebs-Ringers Phosphate solution. Muscle slices 0.5 mm thick were prepared using a Stadie-Riggs slicer and small brain slices approximately 0.5 mm thick were prepared by chopping with a razor blade. The tissue was rapidly washed once with buffer and placed in incubation flasks containing Krebs-Ringer-Phosphate, minimum essential medium (MEM) amino acids minus lysine and arginine, MEM non-essential amino acids, MEM vitamines, and 0.5 mCi each of L-Argine ($3-^3H(N)$, 27.3 ci/mmole and L-lysine ($4,5-^3H(N)$, 55 Ci/mmole (both from New England Nuclear). The slices were incubated at 37° C for 3 hours with continuous shaking under an atmosphere of 95% O_2 - 5% CO_2. Incorporation of radioactivity into protein was linear over the course of the experiment. The incubation was terminated by decanting the buffer, washing the tissue rapidly twice with cold water and homogenizing in 2-4% SDS containing 1% phenylmethyl sulfonyl fluoride (Sigma) to inhibit proteolysis. Aliquots of each homogenate containing 0.1 mg protein were analyzed by SDS-10% polyacrylamide gel electrophoresis according to Laemmli (5) and stained with Coomassie Brilliant Blue. The gel band co-electrophoresing with purified chicken muscle actin run as a marker on a parallel gel was cut out, the radioactive protein eluted in the presence of unlabeled carrier chicken muscle actin and the protein digested with Trypsin-TPCK (Worthington). An aliquot of the digest was then mapped in two dimensions via chromatography and electrophoresis after the method of Gerday, Robbins, and Gosselin-Rey (6) as previously described (7). Autoradiographs of the tritium-labeled peptide maps were prepared according to Randerath (8), following which the chromatography plate was stained with ninhydrin. The criterion of protein identity was then defined as precise coincidence of the autoradiographic spots from the labeled chick muscle or brain actin gel band with the ninhydrin peptides representing the highly purified chicken muscle actin.

RESULTS

The results obtained from the comparison of labeled actin from chick muscle slices with carrier chicken muscle actin are seen in figure 1. Of the approximately 32 ninhydrin peptides, all but one had precisely coincident autoradiographic peptides. There was one autoradiographic peptide with no apparent ninhydrin-sensitive component. Since the muscle slice was labeled with lysine and arginine and the actins were digested with trypsin, theoretically every peptide should be labeled except the c-terminal peptide which should contain neither lysine nor arginine. The results of figure 1 are therefore in agreement with the expected complete identity of muscle actin with itself. The presence of a single autoradiographic spot with no associated

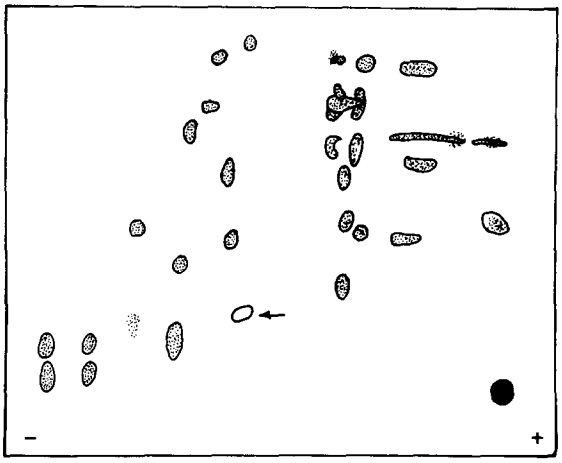

Figure 1. Tryptic peptide map prepared from a mixture of carrier chicken muscle actin and the [^{3}H]Lysine and [^{3}H]Arginine labeled chick muscle actin band from an SDS gel. Ninhydrin-staining material is shown as solid outlines (O) and autoradiographic spots are shown as strippled areas (⊛). Arrow indicates ninhydrin peptide without associated radioactivity. Solid circle at lower right is origin and contains trypsin-insensitive core.

ninhydrin material is probably due to the presence of a ninhydrin insensitive peptide.

Comparison of actin from chick brain slices with carrier chicken muscle actin is shown in figure 2. In this case, there are 2 out of 26 ninhydrin spots without coincident autoradiographic spots. This is one more than the theoretically allowed number for identical proteins. The non-identity of these actins is further emphasized by the presence of six autoradiographic spots without associated ninhydrin material. At least three possible explanations exist for the absence of ninhydrin material from these six radioactive peptides: (i) there are coincident carrier muscle actin peptides which are not reacting with ninhydrin; (ii) the radioactive peptides represent a contaminating protein present in the actin band of the gel from the brain homogenate; (iii) these spots represent additional peptides present in brain actin but not in the muscle actin. The first possibility seems unlikely becuase the peptide map of muscle slice actin with purified muscle actin

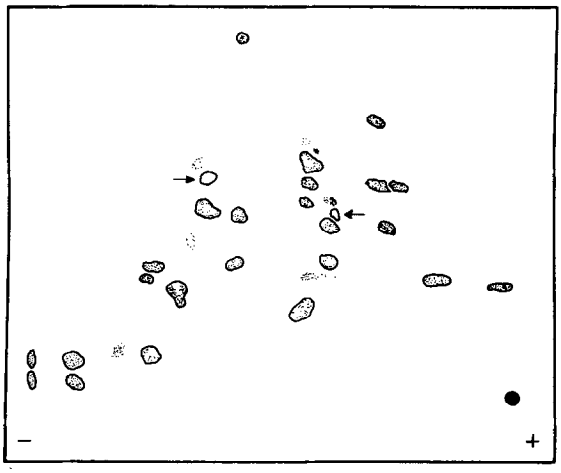

Figure 2. Tryptic peptide map prepared from a mixture of carrier chicken muscle actin and the [^{3}H]Lysine and [^{3}H]Argine labeled chick brain actin band from an SDS gel. Ninhydrin-staining material is shown as solid outlines (O) and autoradiographic spots are shown as stippled areas (❋). Arrows indicate autoradiographic spots without associated ninhydrin-reactive material. Solid circle at lower right is the origin and contains trypsin-insensitive core.

(figure 1) contains only one autoradiographic spot without associated ninhydrin. Since the same carrier actin preparation was used for both maps, this suggests a maximum of one ninhydrin-insensitive peptide, leaving five spots unexplained. The second possibility also seems unlikely since the six autoradiographic spots are of comparable intensity to most of the peptides which do have associated ninhydrin material. Since the postulated impurity would have to be of comparable molecular weight to actin (42,000 daltons) in order to co-migrate with it on an SDS gel and since its tryptic peptides should be labeled with one and only one lysine or arginine, all its peptides should be of roughly the same intensity*. The contaminating protein would therefore contain no more than six lysines plus arginines. In a protein containing about 370 amino acids this small lysine and argine content is

*In fact, the autoradiographic peptides seem to fall approximately into two classes of intensity. This would be consistent with one intensity for lysine-containing peptides and another for arginine-containing peptides assuming different intracellular lysine and arginine pool sizes.

unlikely, although not impossible. The third possibility, that brain actin contains extra peptides not found in muscle actin, therefore seems to us the most likely of the three possibilities.

If non-muscle actins such as brain actin do, in fact, contain extra peptide residues not present in muscle actin, then SDS polyacrylamide gel electrophoresis of sufficient resolution ought to be able to separate a mixture of the two types. Indeed, Kaufman and Roberts (9) have apparently resolved the actin from a continuous line of myoblasts into a doublet on SDS-polyacrylamide gradient gels. Before fusion of the myoblasts, the slower moving (i.e. heavier) actin band in the doublet is dominant while in post-fusion cells the faster migrating band is dominant. These data would be in agreement with our results assuming that pre-fusion myoblast actin is comparable to non-muscle actin.

Our results are quite consistent with those reported by Fine and Bray (4). They found about 10 [^{35}S]methionine-labeled peptides coincident in cytoplasmic and muscle actins, while we have found about 27 coincident peptides using [^{3}H]lysine and [^{3}H]arginine. However, since we are labeling all the peptides, some differences have emerged. In fact, the differences we have reported should be viewed as a lower limit of the actual non-identity because actin has a trypsin-insensitive core, visible on our peptide maps as an intense origin spot. Thus, there may be additional non-identical peptides within this undigested core. It is also probable that the presence of this trypsin-insensitive core accounts for the small variations in the number and pattern of peptides observed in figures 1 and 2 since each individual actin digestion gives slightly different results apparently dependent upon factors such as the physical form of the TCA precipitate and the final concentration of soluble peptides. Winthin any single preparation, however, both the carrier muscle actin and the labeled gel band actin have been subjected to identical conditions since they were mixed together at an early stage of the procedure.

The two ninhydrin spots lacking coincident autoradiographic spots in

figure 2 both have contiguous but non-overlapping autoradiographic spots. Puszkin and Berl (10) have reported that brain actin contains less of the unusual amino acid N^{τ}-methyl histidine than is found in muscle actin (0.3 vs 0.8-1.0 moles/mole), and it is possible that one or both of the slightly displaced brain autoradiographic spots differ from the adjacent carrier actin spots in the methylation of histidines. The non-identity of muscle and non-muscle actins may be due to post-translational modification or, more likely, to their being the products of separate genes.

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