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The Two Components of Spectrin, Filamin, and the Heavy Chain of Smooth Muscle Myosin Show No Detectable Homologies to One Another by Two-Dimensional Mapping of Iodinated Tryptic Peptides*

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SUMMARY. The possible structural relationships among four high molecular weight mechanochemical proteins has been examined using two-dimensional mapping of the tryptic peptide fragments prepared from ^{125}I-labeled proteins (Elder et al., J. Biol. Chem. 252:6510-6515 (1977)). Erythrocyte spectrin bands 1 and 2 protein, the heavy chain of smooth muscle (uterine) myosin, and filamin from human and rabbit were studied. The maps of the four proteins within each species differed considerably from each other, with no apparent homologies evident among them, whereas maps of the same individual protein between the two species showed a high degree of homology.

There exist a number of large mechanochemical proteins within different cells in a given species whose relationship to one another is obscure. These proteins all have uncommonly large polypeptide chains (molecular weight >$2x10^5$ daltons) and share the ability to interact with actin. They include the following four proteins: the two components (bands 1 and 2) of erythrocyte spectrin (1), the heavy chain of smooth muscle myosin, and filamin (2). Although these proteins are all clearly different from one another, the possibility of structural relationships among at least some of them has been suggested (see Discussion). Very little detailed structural information about these proteins exist at present, however, due primarily to the difficulty of applying the classical techniques of protein chemistry to polypeptide chains of such large size. A rapid survey of possible structural relationships among them is now

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feasible, however, through the application of recently developed methods (3, 4) of two-dimensional mapping (fingerprinting) of tryptic peptide from proteins that are ^{125}I-iodinated in SDS-polyacrylamide electrophoresis gels. Such methods have two important attributes. First, since only a fraction of the total set of tryptic peptides contain iodinatable residues and can therefore be visualized by autoradiography, only a manageable number of peptide spots are observed on the maps even for large polypeptide chains. Second, the method is so sensitive that the protein from a band on a single slab or cylindrical gel is enough for analysis, thereby obviating the need to first purify each of the proteins in question. (Preliminary purifications were nevertheless carried out in these experiments, see following section). In this communication, we report such fingerprinting of the proteins mentioned from both human and rabbit sources. No detectable homologies were found among the maps of the four proteins within a single species, whereas extensive homologies were observed for a given protein between the two species.

MATERIALS AND METHODS

Proteins. Spectrin was prepared from erythrocyte ghosts by the method of Fairbanks et al. (5). Filamin was extracted from WI38 human fibroblast cultures (6) by perfusion of frozen and thawed cells in a buffer containing 0.3 M KCl, 0.05 M histidine, 2 mM ATP, at pH 6.8, followed by precipitation of the extract with 35% saturated $(NH_4)_2SO_4$. The precipitate was then dissolved by dialysis into a buffer containing 0.05 M KCl, 0.05 M histidine, pH 7.0. Smooth muscle myosin was prepared from uteri by published methods (7). All of these proteins were then subjected to electrophoresis in SDS on 4-15% polyacrylamide gels following the methods of Laemmli (8). Spectrin bands 1 and 2 were sliced out of the same gel.

Peptide Mapping. The procedures used for iodinating the gel slices, trypsinization, and two-dimensional separation of the peptides were those of Elder et al. (4) with the following changes. Gel slices were iodinated in 10 x 75 mm tubes which had been siliconized by treatment with a 1% solution in benzene of dimethyl dichlorosilane (Sigma). Unconjugated iodine was removed by washing the slices in their iodination tubes with 10% methanol, changed once a day for three days. Lyophilization was used for all drying procedures. Peptide mapping was carried out on 20 x 20 cm cellulose-coated glass TLC plates (EM labs). Usually only 1 cpm per unit molecular weight of protein was applied to the plate, and this was compensated for by enhancing the efficiency of autoradiography, using for the latter Kodak BB-5 X-ray film and DuPont Chronex "Hiplus" intensifying screens at -70°C with exposures for 24 hrs. For two-dimensional mapping, electrophoresis proceeded until the tracking dye (TLC tracking dye, Scientific Products) had migrated 8 cm; chromatography employed buffer II of Elder et al. plus 7% 2, 5 dipenyloxazole (PPO), and proceeded un-

til ε-DNP lysine (used for tracking) had migrated 11 cm. With these changes, a substantial reduction in background and improved resolution of the ^{125}I-labeled peptide spots was achieved.

RESULTS

The verification of the peptide mapping procedure, its reproducibility and its calibration with cytochrome C and serum albumins of known amino acid sequences will be detailed elsewhere (S. E. Zweig and S. J. Singer, to be published).

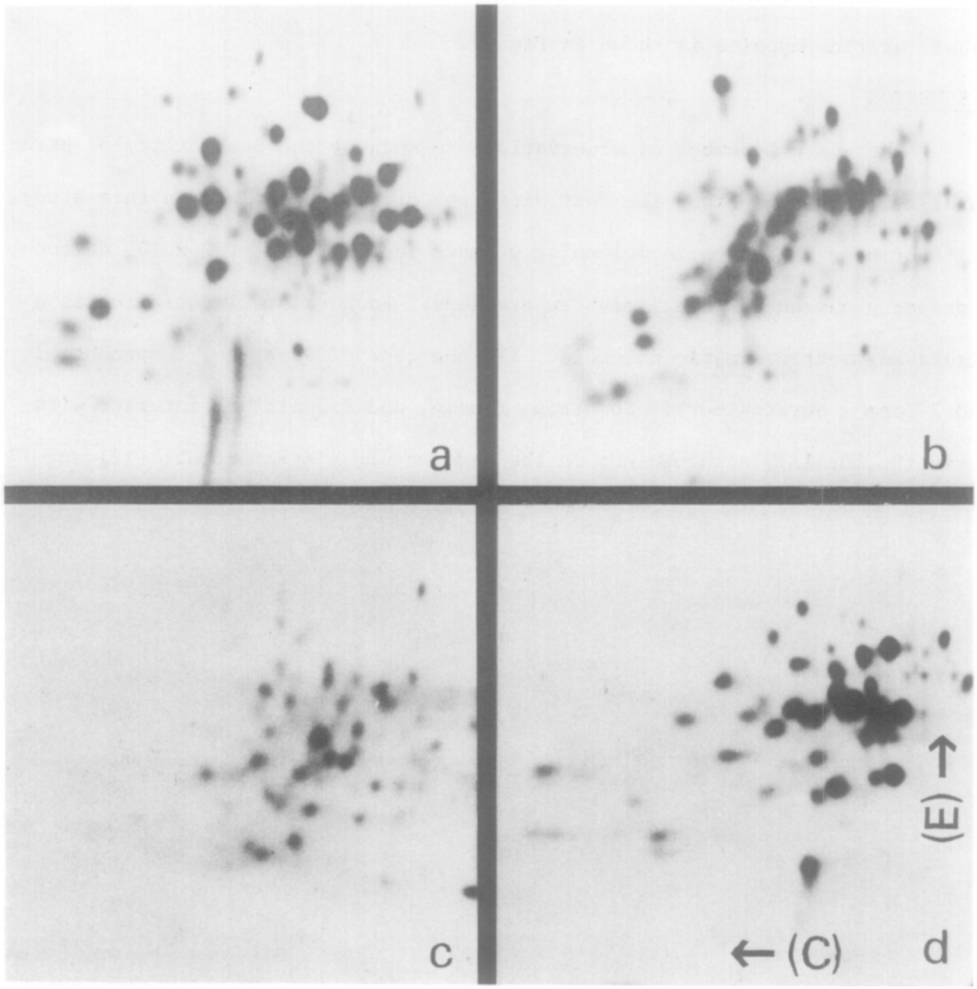

Figure 1

Maps of human spectrins, fibro last filamin, and smooth muscle myosin: a, spectrin band one; b, spectrin band two; c, WI38 fibroblast filamin; d, uterine myosin heavy chain. Directions of electrophoresis (E) and chromatography (C) are shown in 1d. Origin is in the lower right hand corner. 2.5 x 10^5 cpm, 22 hr exposure.

Representative peptide maps of human spectrin bands 1 and 2, uterine myosin, and fibroblast filamin, are reproduced in Fig. 1. The complexity of all four maps is comparable, each showing approximately 50 ± 10 spots. Variations in the specific activity of the peptides produce artifactual variations in size as determined by autoradiography. Superposition of these maps show no obvious peptide similarities among them beyond what would be expected by chance overlap of spots. By contrast, maps of each of the individual proteins from the two different species show substantial overlap; the example of human and rabbit uterine myosins is shown in Fig. 2.

DISCUSSION

There are a number of observations to suggest the possibility of structural relationships among the four different proteins examined in this study. All four have single chain molecular weights in the range of 2×10^5 daltons, a property shared by relatively few proteins. Each exists in solution as a highly asymmetric dimeric molecule. (In the case of spectrin, components 1 and 2 form a heterodimer). Spectrin, myosin, and filamin all interact with actin (9, 10, 11), although not in the same ways. Bjerrum et al. (12) sug-

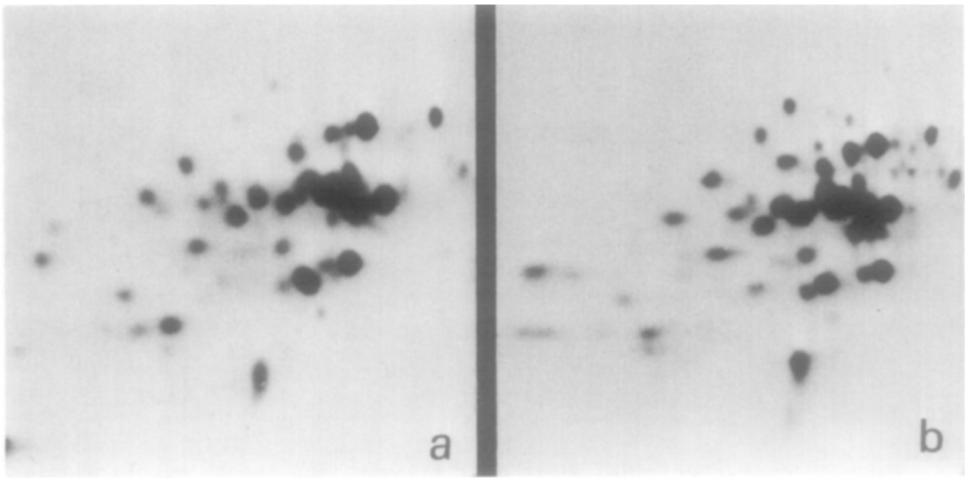

Figure 2
Maps of smooth muscle myosin from two different species. 2a, rabbit uterine myosin. 2b, human uterine myosin. 2.5×10^5 cpm, 24 hr.

gested that human spectrin components 1 and 2 cross-react antigenically, but others (13, 14) have found no such cross-reaction. Sheetz et al. (13) found a weak antigenic cross reaction between human uterine myosin and spectrin. Studies by Wang et al. (15) have detected no antigenic cross-reaction between chicken gizzard filamin and myosin, and other studies by Wallach et al. (16) did not find any cross-reaction between guinea pig vas deferens filamin and spectrin. While the immunochemical data mentioned above suggest that few if any homologies exist among these proteins, it is always possible that those regions of the molecules which were homologous were antigenically silent. As a preliminary to detailed structure or sequence information, we report here a survey of the ^{125}I-labeled tryptic peptide maps derived from these proteins. These maps show these proteins to be substantially different from one another, and provide no evidence that they are related. Both human and rabbit spectrin components 1 and 2 are, somewhat surprisingly, apparently as different from one another by this technique as either is from myosin and filamin. These findings differ from the results of Maddy and Dunn who reported a marked similarity between the two components of bovine spectrin using an alternative technique of tryptic peptide mapping (17). Although the possibility of species differences can not be ruled out at this time, we believe that the lower resolution of their technique as well as a lack of comparison between spectrins and suitable reference proteins (to determine the extent of nonspecific map homologies) could have led to an overestimation of the sequence homology between components 1 and 2.

The conclusions from these experiments are, however, limited. Our experience with reference proteins of known amino acid sequence has shown that the iodinated tryptic peptide fingerprint method is capable of detecting the relatedness between two proteins only if there is substantial sequence homology between them. It is difficult to give quantitative expression to this limitation, but it appears likely that if sequence homology exists but is less than 50%, the iodinated tryptic fingerprint method would not detect it. We

therefore cannot rule out the existence of such lesser homologies among the proteins examined in this study, but if they do exist, it will require detailed amino acid sequencing to reveal them.

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