PROTEOLYTIC DIGESTION PATTERNS OF SPECTRIN SUBUNITS

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1. Introduction

Spectrin, which is the most abundant protein associated with the erythrocyte membrane, and is evidently involved in maintaining the shape of the cell [1], contains two kinds of subunit, tightly associated with each other [2], in equimolar proportions. These chains have approx. mol. wt 240 000 and 220 000. It has been suggested [3–6] that spectrin may resemble myosin, though the only direct argument for such a view is its reported immunological cross-reaction with the myosin of a smooth muscle [6]. In chemical terms, little is known about the relationship between the two spectrin subunits, and one possibility which must be considered is that one is derived by post-synthetic proteolysis of the other. To establish whether two species are derived from the same gene, originate from genes of common ancestry, or are totally unrelated, presents problems for proteins of such high molecular weight. Conventional peptide maps in particular will be expected to contain too many spots to permit of quantitative comparisons. Papain can be used to accomplish partial degradation of proteins into fragments in a desired size range, in the presence of the dissociating agent, sodium dodecyl sulphate (SDS) [7]. A useful variant of this procedure consists in performing the digestion on an electrophoretic zone cut from an acrylamide gel. This ensures that the starting material is pure, and obviates problems of isolation. The gel slice is then reintroduced as the sample on an acrylamide gel, and electrophoresis is performed as before. Breakdown patterns thus displayed can be compared with great precision. We describe here the results of such a comparison between the two spectrin subunits. We have also compared the patterns of peptides generated by cyanogen bromide cleavage [8]. The results show at once that the sequence of the two chains are different, but there is clear evidence that they are nevertheless related, and therefore presumably share an ancestral gene. A comparison with myosin and with the cytoplasmic high molecular weight filamentous protein, filamin [9], is also shown.

2. Materials and methods

Spectrin was prepared and purified as in [2,10,11]. Myosin from rabbit skeletal muscle was prepared as in [12] and filamin from chicken gizzard as in [13]. Papain was obtained from Sigma Chemical Co.

The first electrophoresis was performed in each case on a slab gel in the presence of SDS, using a discontinuous system [14] and 5% acrylamide. Samples were applied in slots of 8.5 mm width. Bands of protein, lightly stained with Coomassie brilliant blue G200, each containing about 70 μg protein, were cut from the gel, soaked in stacking gel buffer for the second gel system and kept at −20°C [7]. The papain fragments were separated in a slab gel 9 cm length, containing a linear gradient of 5–20% acrylamide, with a 5% stacking gel 7 cm length set over the top. The buffer system was that in [14]. Papain was dissolved in sample buffer, containing additionally 20% glycerol, 2% β-mercaptoethanol, 0.1 mM EDTA and a trace of bromophenol blue. The optimal papain concentration was found to be about 0.1 μg/ml, and 5 μl of this solution was carefully introduced into each sample slot. The gel containing the protein sample was then placed on top, and electrophoresis

was begun. When the bromophenol blue marker had migrated 2–3 cm through the stacking gel, the current was switched off for 30 min. Electrophoresis was then performed overnight at 6 V/cm (1.2 kV h total). The gel was fixed in 10% trichloroacetic acid and stained with Coomassie brilliant blue G200 as in [14]. Densitometric scanning was performed in a Joyce-Loebl instrument, using a red filter. Migration distances were measured both directly from the gel and from the densitometer traces.

3. Results and discussion

Typical gel electrophoresis patterns of fragments generated by the action of papain on the two spectrin components are shown in fig.1. It is clear that the two patterns are far from identical, and this is also true of similar results generated after more extensive digestion, using higher papain concentrations. We can therefore at once conclude that the two components do not share the same sequence, and are thus not

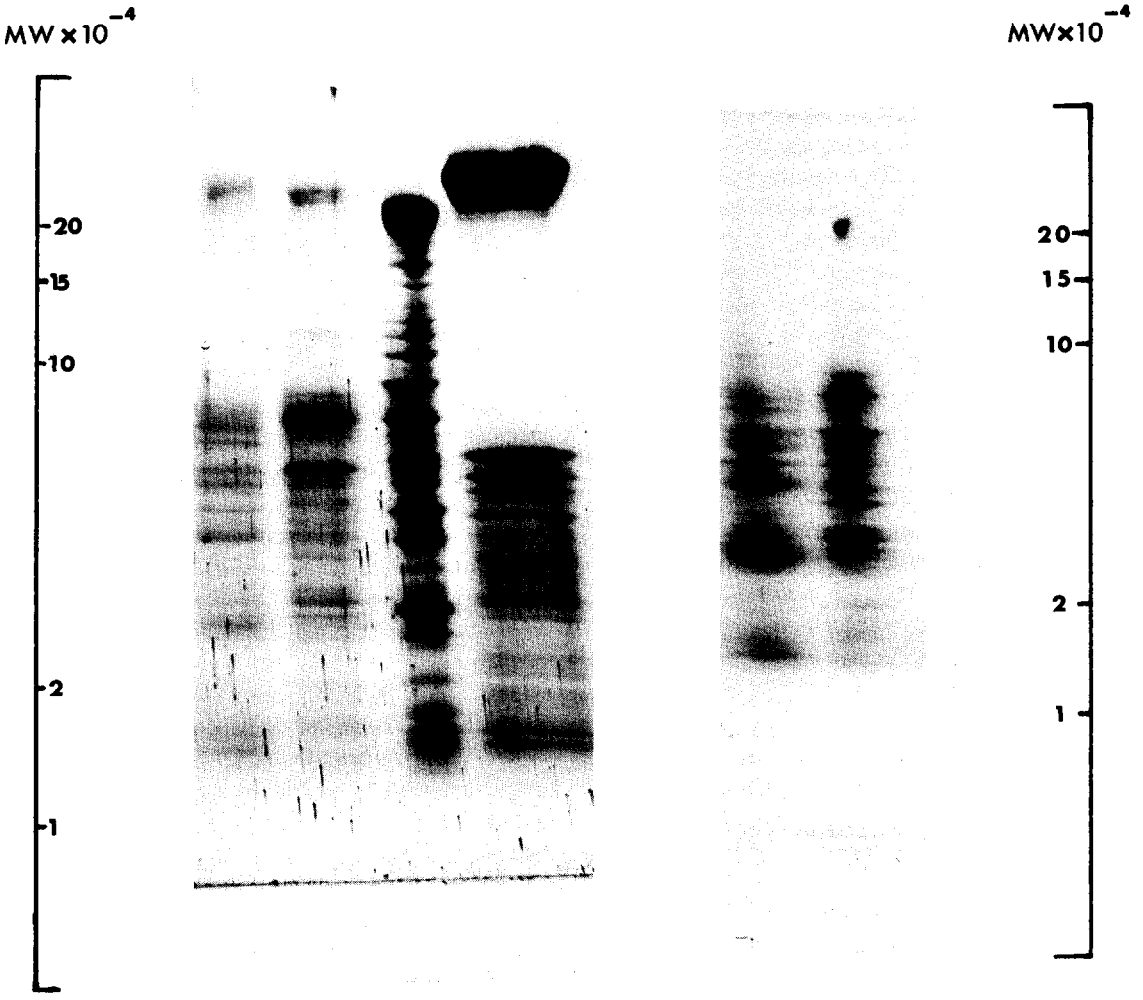

Fig.1. Polyacrylamide gel electrophoresis (5–20% gradient), in the presence of SDS, of papain digestion products of (left to right): spectrin (larger subunit), spectrin (smaller subunit), skeletal muscle myosin, chicken gizzard filamin. The right-hand panel shows a separate experiment, with only the two spectrin subunits. The approximate molecular weight scales are based on marker proteins.

related through a post-biosynthetic degradation of the longer chain. At the same time several common constellations of zones are evidently present in the two patterns. An attempt may be made to express the degree of similarity quantitatively, using a procedure devised earlier for the analysis of nucleolytic fragmentation patterns of RNA [15]. By taking account of the electrophoretic resolution, one may divide the part of the gel containing the pattern of zones to be compared into N contiguous equal compartments, each of which may be empty or may contain a zone. In comparing two patterns, one of n and the other of m zones, distributed among the N compartments, the probability of any number of coincidences, x, may be readily determined. This probability $P(x)$ is subject to the condition that $\Sigma P(x) = 1$, and is given by

$$P(x) = m!n!\,(N-m)!\,(N-n)!\,/N!x!\,(m-x)!\,(n-x)!\,(N-m-n+x)!$$

Thus if x is sufficiently large to make $P(x)$ very small, it is very improbable that such a degree of coincidence could come about by accidental matching of zones. In the typical patterns shown for the two spectrin components of fig.1, $N = 140$ (taking the resolution as 0.5 mm), $n = 20$, $m = 23$, and x, the number of zones of identical mobilities within the discrimination of the system, is 11. $P(x)$ is then 1×10^{-5}, which effectively excludes the possibility that this degree of matching may have come about by chance. Patterns of peptide fragments have also been generated by cleavage at methionine residues with cyanogen bromide. There are fewer zones, but a degree of similarity again exists.

Figure 1 also makes possible a comparison of the digestion products of the two spectrin chains with those of myosin heavy chains, and chicken gizzard filamin. No visual similarity is obvious but statistical analyses have been performed. A common criterion for a valid correlation is $P(x) < 0.01$, and on this basis our results do not give evidence in favour of any significant sequence similarities between the spectrin subunits and filamin, although of course short-range homologies are impossible to rule out. As regards myosin, a very large number of fragments is produced and this vitiates statistical analysis because n approaches too closely to N. The results do not rule out a degree of homology, but on the other hand give no evidence in its favour. Any homologies are certainly (as visual inspection shows) not close ones. Studies are now required to compare spectrin with other high molecular weight proteins occurring in the cytoplasm of eukaryotic cells, and thought to be involved in translocational processes (reviewed [16]).

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