Vol. 132, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1985 Pages 382-389

COMPARISON OF SYNEXIN ISOTYPES IN SECRETORY AND NON-SECRETORY TISSUES

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Received August 28, 1985

Three synexin isotypes were identified in bovine liver or adrenal medullary tissues by immune blotting of one- or two-dimensional SDS gels and by two-dimensional tryptic peptide mapping of gel bands or spots. These isotypes were: α-synexin, mass 47 kDa, pI 6.9 ; β-synexin, mass 47 kDa, pI 6.5; and μ-synexin, mass 51 kDa, pI 6.1. A non-secretory tissue, bovine skeletal muscle, was found to contain only μ-synexin. The absence of α-and β-synexins in a non-secretory tissue suggests these proteins may perform specific roles in the process of exocytosis.

Synexin is a 47 kDa Ca^{2+} binding protein that causes the Ca^{2+}-dependent aggregation of isolated chromaffin granules in vitro (1). It has been hypothesized that synexin may also promote membrane contact and fusion in secretory cells, acting as an intracellular receptor for Ca^{2+} in the process of exocytosis. (1). The protein has been detected in several tissues, including adrenal medulla, adrenal cortex, brain, spleen, liver and parotid, suggesting it may play a fairly general role in membrane fusion events (2). In this communication, we report the discovery of a 51 kDa synexin variant that was first identified as a minor band on immune blots of partially purified liver and adrenal synexins. This new variant was found to be the only form of synexin present in skeletal muscle, from which we have isolated the protein and characterized its chromaffin granule aggregating properties.

0006-291X/85 $1.50

MATERIALS AND METHODS

Preparation of Tissue Fractions and Synexins. Cytosol fractions (post microsomal supernatants) were prepared from 250 gms of bovine skeletal muscle orliver orfrom 100 gms of adrenal medullary tissue homogenized in 2 volumes of 0.3 M sucrose, 40 mM MES (pH 6.0), as previously described (1) A crude, synexin-containing fraction used for immune blotting (fig.1) was obtained by precipitation from this supernatant in 20% ammonium sulfate. Synexin was purified from this precipitate by repeated precipitation in 20% ammonium sulfate and gel filtration on LKB Ultrogel AcA 34 (1). Chromaffin granules were prepared by differential centrifugation in 0.3 M sucrose (1).

Analytical Procedures.

Protein electrophoresis was performed in one dimension according to Laemmli (3) or two dimensions according to O'Farrell (4), as detailed previously (5). Immune blotting was performed as described by Burnette (6), using ^{125}I labeled protein A and autoradiography to detect antibody-binding bands on the nitrocellulose blots (6). The goat antiserum to bovine liver synexin was prepared as described (5). Two dimensional tryptic peptide maps of gel bands or spots were prepared as described by Elder, et al. (7). The chromaffin granule aggregating activity of synexin was assayed as described (1) in the presence of a Ca/ EGTA buffer (8). The value of 4.92×10^4 was used as the binding constant of EGTA for Ca^{2+} at pH 6.0(8). Protein was determined according to Bradford (9).

RESULTS

When the antiserum to synexin was used to detect synexin on an electrophorectic blot of proteins precipitated from liver cytosol in 20% ammonium sulfate two protein bands were detected: A major one of apparent mass 47 kDa; and a minor one of mass 51 kDa (Figure one, lane "L"). A similar pattern was observed when a crude ammonium sulfate fraction from adrenal medullary tissue was tested (not shown). In previous reports on the properties of synexin isolated from either the adrenal medulla or liver (10), the most highly purified preparations of this protein were usually seen to contain a minor 51 kDa "contaminant" in addition to the major 47 kDa species. The ratio of the staining of these two bands was about 10:1, comparable to the ratio of staining intensity of the crossreacting bands seen in the immune blotting experiment (figure 1). In contrast, however, when a similar 20% ammonium sulfate cut was prepared from a cytosolic extract of a non-secretory tissue, skeletal muscle, only the 51 kDa band was

detected (Figure 1, lane "M"). When liver and muscle extracts were co-electrophoresed, the 51 kDa dalton proteins from the two sources were found to have the same electrophoretic mobility (figure 1, lane "L+M").

It was possible to isolate synexin from skeletal muscle by application of the conventional procedures: Repeated precipitation in 20% ammonium sulfate and gel filtration (figure 2). However, the yield of synexin (~ 1 μgm/gm of tissue) was only about 10 to 20% of the yield obtained from liver or adrenal

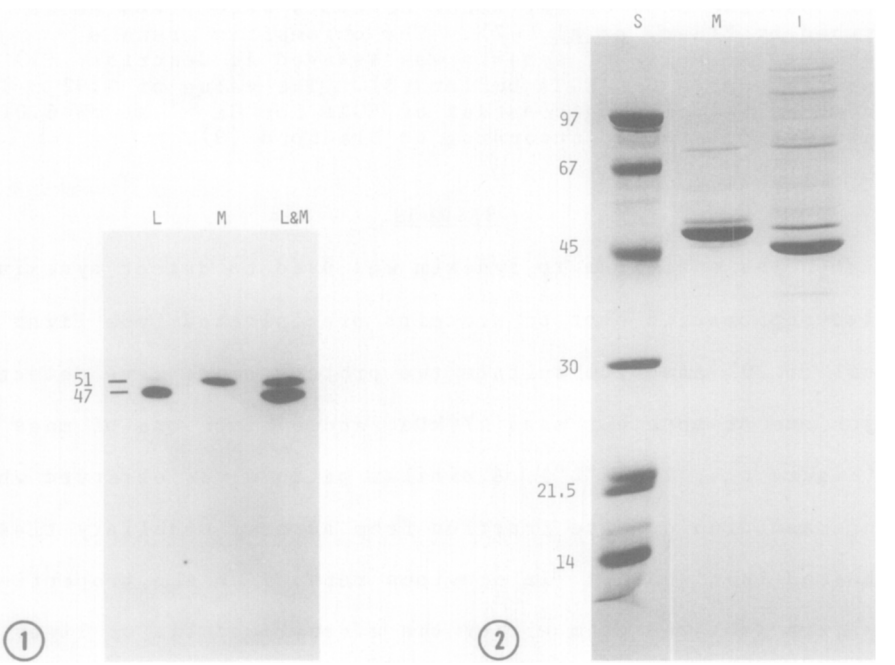

Figure 1. Detection of synexin isotypes in liver and muscle extracts by immune blotting of one-dimensional SDS gels. Crude extracts prepared from liver ("L") or muscle ("M") as described in Materials and Methods were applied individually, or as a mixture ("L + M") to a 10% polyacrylamide gel. After electrophoresis and transfer to nitrocellulose, the synexin was detected with a specific antiserum and ^{125}I-labeled protein A. In the autoradiograph shown, synexin isotypes are detected at 47 kDa ("47") and 51 kDa ("51").

Figure 2. SDS gels of preparations of synexin from skeletal muscle ("M") and from liver ("L"). The molecular weights (x 10^{-3}) of standard proteins ("S") are indicated on the left.

medullary tissues. The major protein in the muscle synexin preparation had a mass of 51 kDa (fig. 2).

The isolated liver synexin was subjected to electrophoresis in two dimensions to determine the isoelectric points of the cross reacting isotypes. As seen in figure 3 ("L") the 47 kDa protein band consists of two major species of isoelectric points 6.9 (80%) and 6.5 (20%). In addition, the 51 kDa isotype appeared to have an isoelectric point of 6.1. When the isolated muscle synexin was similarly analyzed (figure 3, "M"), the 51 kDa protein was found to have identical electrophorectic parameters to the 51 kDa isotype from liver. In this gel a trace amount of the liver preparation was included to provide markers for the positions of the various isotypes; the 47 kDa species, as indicated above, did not arise from the muscle preparation. We have chosen to refer to these isotypes

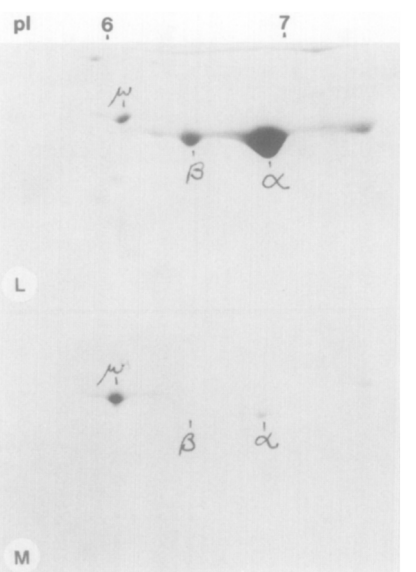

Figure 3. Resolution of synexin isotypes by 2-dimensional electrophoresis. In the upper panel ("L") liver synexin is resolved into 3 isotypes labeled α, β, μ. An approximate scale of isoelectric points is indicated at the top ("pI"). In the gel represented in the bottom panel ("M"), muscle synexin was combined with a small amount (~10%) of liver synexin to mark the positions of the α and β isotypes.

of synexin as α , the major 47 kDa form (PI 6.9); β , the minor 47 kDa form (PI 6.5); and μ (for "muscle"), the 51kDa form (pI 6.1).

In order to determine if the crossreacting 47 kDa and 51 kDa synexin isotypes also have similar primary structures, two dimensional maps were prepared of iodinated tryptic peptides obtained from the two proteins after elution from one-dimensional SDS gel (figure 4,a and b). Although this technique is highly sensitive to minor differences in primary structure (7), no significant differences could be seen between the two maps. For comparison, maps were also prepared of bovine serum albumin and of 67 kDa bovine calelectrin (11), ("synhibin", ref.12), both of which also interact with Ca^{2+} and

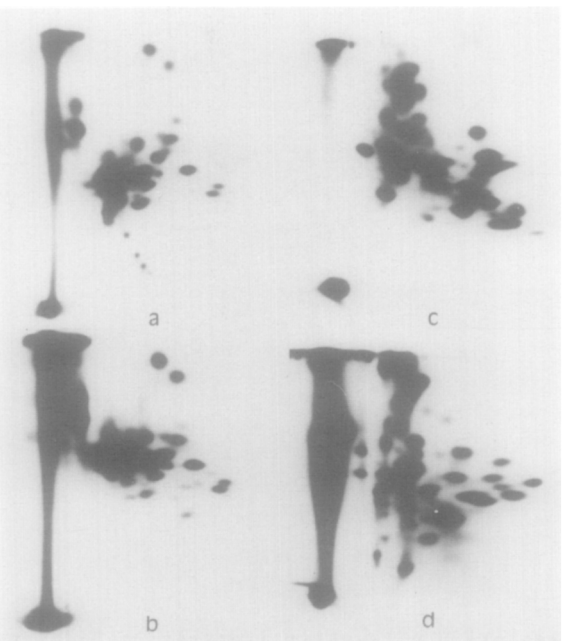

Figure 4. Two dimensional maps of tryptic peptides from liver synexin (a),muscle synexin (b), 67 kDa calelectrin ("synhibin") (c), and bovine serum albumin (d). The origin for each map is at the lower left; electrophoresis was in the horizontal direction, chromatography in the vertical.

with lipids. These maps were easily distinguished from one another and from the synexin isotypes (figure 4, c and d). The mass difference between the two synexin isotypes is apparently not reflected in the mobilities of the particular peptides seen in these maps.

When a 2-dimensional gel of liver synexin was analyzed in the immune blot procedure, all 3 isotypes of synexin reacted with the antiserum (not shown). Since the antigen used to prepare this antiserum was a 47 kDa band eluted from a one-dimensional gel (5), the α and β forms of the protein might in fact be unrelated, since they were both present in the immunizing antigen. However, 2 dimensional peptide maps similar to those in figure 4 were prepared from each isotype excised from a 2-dimensional gel. These maps were indistinguishable from one another or from the synexin maps presented in figure 4. The α and β forms appeared not to be related to one another by phosphorylation since prolonged digestion of synexin with alkaline or acid phosphatases did not eliminate the β isotype (W.J. Zaks and C.E. Creutz, unpublished observation).

Since only μ-synexin can be extracted from skeletal muscle, it was possible to analyze the chromaffin granule aggregating properties of this specific isotype and compare them with the properties of the 47 kDa form(s). Both liver and muscle synexin were found to aggregate chromaffin granules, and their dependencies on Ca^{2+} were virtually identical (figure 5). Similar amounts of each preparation were required to promote granule aggregation (3 to 5 μg protein in the presence of 130 μg granule protein) so the granule aggregating activity of the liver synexin prep could not be attributed to the small amount ($\leq$ 10%) of μ synexin present in liver.

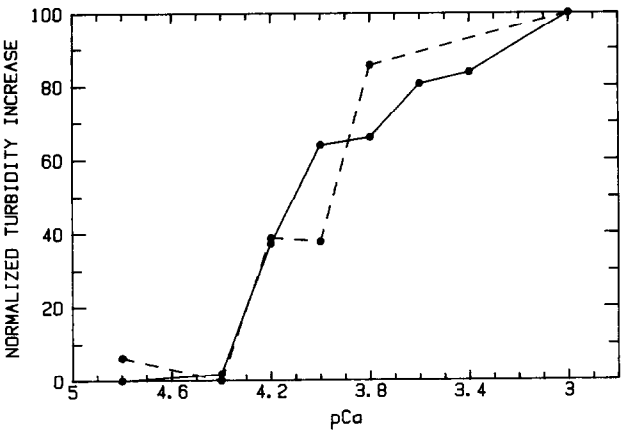

Figure 5. Ca^{2+}-dependence of the aggregation of chromaffin granules by liver synexin (solid line) and muscle synexin (dashed line). pCa = - log $[Ca^{2+}]$. Normalized turbidity increase is the increase in absorbance at 540 nm of a chromaffin granule suspension (~130 μg/ml) after a 10 min incubation with ~5 μg of synexin. The data for each protein were separately normalized to 0 for the turbidity of the suspension after a 10 min incubation without synexin, to 100 for the turbidity after a 10 min incubation with synexin at pCa 3.0.

DISCUSSION

Although synexin was originally discovered in extracts of adrenal medullary tissue (1), it has since been detected in a wide variety of tissues (10) including brain, liver, parotid, spleen, adrenal cortex, and, now, skeletal muscle. It has been purified only from adrenal medulla, liver and muscle. This wide tissue distribution suggests it must possess a more general function than inducing only the fusion of chromaffin granules. It is possible it is involved in Ca^{2+}-dependent membrane contact and fusion events in general. However, it would still seem likely that the content or properties of synexin in a distinctly non-secretory tissue such as skeletal muscle would differ from those of synexin from secretory organs such as the adrenal medulla or liver. Indeed, as indicated here, the two major isotypes of synexin of mass 47 kDa that are present in secretory tissues appeared to be completely absent from muscle

tissue. The minor 51 kDa isotype proved to be universal. In fact, judging on the basis of the yield of synexin from muscle, the amount of 51 kDa isotype is similar in all tissues examined. It is possible that this isotype arises from some cell type common to all tissues, such as connective tissue or vascular cells. Detailed localization studies, or extraction from cloned cell types may be necessary to resolve this question. The absence of the 47 kDa synexin species from non-secretory tissue provides additional evidence that these forms of the protein may play specific roles in the process of exocytosis.

ACKNOWLEDGEMENT

This study was supported by grants from the National Science Foundation (PCM 8206453) and the National Institutes of Health (AM 33151).

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