# LYMPHOCYTE α-ACTININ

## Relationship to Cell Membrane and Co-capping with Surface Receptors

DANIEL HOESSLI, ELISABETH RUNGGER-BRÄNDLE, BRIGITTE M. JOCKUSCH, and GIULIO GABBIANI

From the Department of Pathology, School of Medicine, University of Geneva, Geneva, Switzerland, and the European Molecular Biology Laboratory, D-69 Heidelberg, German Federal Republic

ABSTRACT

Mouse spleen lymphocytes synthesize a protein which comigrates with skeletal muscle α-actinin on two-dimensional gel electrophoresis and is immunoprecipitated by an antibody directed against skeletal muscle α-actinin. Mouse lymphocyte α-actinin is present in membrane fractions, and is immunoprecipitated from lymphocyte detergent lysates by an antiserum made against these purified membranes. The anti-α-actinin activity of this antiserum is not adsorbed after incubation with fixed intact lymphocytes. Lymphocyte α-actinin does not bind concanavalin A and it is inaccessible to lactoperoxidase-catalyzed surface iodination. Double immunofluorescence shows that α-actinin moves concurrently along the cell membrane with redistributed surface immunoglobulins and Thy-1 antigen, and remains associated up to 30 min with surface aggregates of these receptors. Our results suggest that lymphocyte α-actinin, as defined by molecular weight and cross reactivity with the antibody against the muscle protein, (*a*) is associated with the cell membrane, (*b*) is not expressed at the cell surface, and (*c*) participates in the movement of surface receptors.

KEY WORDS cell membrane · cytoskeleton · microfilaments · surface receptors

Redistribution of surface receptors has been used as an experimental model for studying the relationships between cytoplasmic components and membrane structures in contact with the external environment (1, 2, 5, 11, 20, 39, 49). Immunochemical and ultrastructural observations indicate that cytoskeletal proteins accumulate under ligand-induced aggregates of surface receptors (6, 14, 41, 51, 52); the process of ligand-induced redistribution of receptors is sensitive to drugs affecting the structure or function of cytoskeletal proteins (46, 49). The association of actin with shed surface components has been demonstrated for cells in suspension (13, 28). Thus, surface receptors and cytoskeletal proteins appear to be capable of interaction through the hydrophobic portion of the plasma membrane.

α-Actinin, one of the structural proteins of striated muscle Z-line, is present in nonmuscle cells. In fibroblasts, platelets, and the terminal web of intestinal epithelial cells, it has been suggested that α-actinin is present at the attachment sites of microfilaments to the plasma membrane (7, 9, 16, 32). The possibility that α-actinin represents a necessary element to join membrane and cytoskeleton in lymphocytes has been suggested (5, 35). The capacity of α-actinin to interact with actin

in vitro (18, 47) and its frequent association with plasma membranes (7, 16, 32) make it a likely candidate for this function.

In this paper, we have characterized lymphocyte α-actinin by immunoprecipitation and two-dimensional (2-D) gel electrophoresis, and documented its presence in light density membrane preparations. Furthermore, we have shown that α-actinin moves concurrently along the cell membrane with redistributed surface immunoglobulins and Thy-1 antigen.

## MATERIALS AND METHODS

### *Animals and Cells*

8- to 10-wk-old DBA/2 mice were killed by cervical dislocation, and their spleens were aseptically removed and teased apart in Hanks' balanced salt solution (HBSS). Contaminating erythrocytes were lysed in $NH_4Cl$ 0.75% (wt/vol), Tris-HCl 20 mM, pH 7.4. Lymphocytes and nucleated cells were further washed in HBSS. Floating small lymphocytes were separated from larger cells on 5–15% isotonic sucrose gradients. Over 90% viable small lymphocytes were obtained with this procedure and used for biosynthetic labeling and immunofluorescence staining. Balb/c P1798 lymphoma cells (Litton Bionetics Inc., Kensington, Md.), carried in Balb/c mice as ascites tumors, were used for membrane preparation.

### *Electron Microscopy*

Membrane fractions were fixed in 1.8% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h at room temperature, washed for several hours in cacodylate buffer, postfixed in 1.3% $OsO_4$ for 30 min at 4°C, stained en bloc with 2% uranyl maleate, pH 6.0, for 1 h at 4°C, then rapidly dehydrated in ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

### *α-Actinin Extraction and Preparation of an Anti-α-Actinin Antibody*

α-Actinin was extracted from porcine skeletal muscle and purified on hydroxyapatite (50). Such a preparation has an apparent mol wt of 100,000 (Fig. 1*a*), and, like actin, focuses with an apparent pI of 6.4 in the middle of our pH gradient in the O'Farrell system (40).

Anti-α-actinin antibody was obtained in rabbits by injecting the purified protein precipitated with alum (23, 32), affinity-purified on α-actinin-Sepharose from an immunoglobulin fraction, and eluted from the absorbent with 4 M $MgCl_2$. By immunoperoxidase staining (53) of a crude α-actinin preparation, the purified antibody detects only the 100,000 component (Fig. 1*b*); faint staining of bands in the region of ~80,000 reflects proteolytic cleavage products of α-actinin. This band is a breakdown product of α-actinin, since successive reruns of gel-eluted 100,000 α-actinin always reveal an 80,000 contaminant. The antibody does not show any affinity for high molecular weight contaminants or for actin.

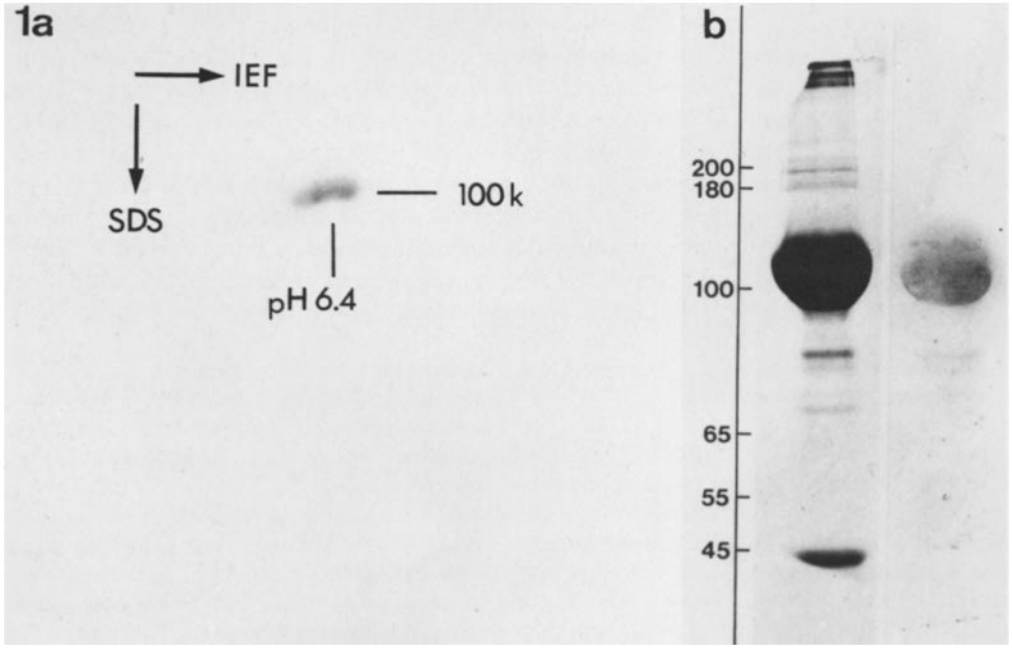


FIGURE 1 Characterization of skeletal muscle α-actinin used as immunogen and of affinity purified anti-α-actinin antibodies. (*a*) 2-D gel electrophoresis of the α-actinin preparation; 7.5% polyacrylamide gel was used for the second dimension; Coomassie blue staining. (*b*) Left: SDS-polyacrylamide gel of a crude α-actinin preparation (50 μg); right: immunoperoxidase staining of this preparation with affinity-purified anti-α-actinin antibodies.

### *Anti-concanavalin A (Con A) Antibodies*

A rabbit was injected twice at a 15-d interval with Con A (type V, Sigma Chemical Co., St. Louis, Mo.) and bled 10 d after the last injection. Anti-Con A antibodies were purified by affinity chromatography of the antiserum on Con A-Sepharose, in the presence of 0.1% (wt/vol) α-methylmannoside. Specific antibodies were eluted with KSCN and dialyzed against phosphate-buffered saline (PBS).

### *Cell Fractionation and Preparation of an Anti-Membrane Antiserum*

P1798 Lymphoma cells were obtained from pooled ascites fluids and extensively washed in PBS. Homogenization was performed by mechanical disruption using a Stansted AO 612 cell disrupter equipped with a 516 valve (Stansted Fluid Power Ltd, Stansted, England). Membranes were obtained by flotation in sucrose, starting from a crude membrane pellet (10). Alternatively, cells were homogenized in 60% sucrose with a glass Teflon Potter homogenizer, and pure membranes were obtained by flotation without prior pelleting of the crude membrane fraction (36, 37). Both methods gave floating membrane fractions of similar purity as judged by electron microscopy. The second method, however, gave higher yields of the top fraction lighter than 22.5% sucrose. The two lightest fractions were analyzed for their protein content on 7.5% polyacrylamide slab gels (30).

The ultrastructural analysis of the fraction collected between the overlaying buffer and 22.5% sucrose is shown in Fig. 2 *a*. Membrane vesicles, free of cytoplasmic content, are the only structures detectable in this preparation. No attached fuzzy material is visible even at higher magnification. The second floating fraction (between 22.5 and 35% sucrose) is morphologically identical to the first fraction. Fig. 2 *b* shows SDS-polyacrylamide gels of both fractions after extraction in SDS sample buffer. In both fractions, a band comigrates with skeletal muscle α-actinin; another band of lower mol wt, present also in both fractions, comigrates with skeletal muscle actin.

A mixture of the two floating fractions described above in complete Freund adjuvant totalling 400 μg of proteins was injected intramuscularly to rabbits twice at a 15-d interval. Rabbits were bled starting 2 wk after the last injection.

To prepare anti-cell surface antibodies, an immunoglobulin fraction of the anti-membrane antiserum was incubated with P1798 cells lightly fixed with formaldehyde. The adsorbed antibody was eluted by acid treatment of the fixed cells, following the procedure of Yamana et al. (54). Anti-cell surface antibodies bind exclusively to lymphocyte products that are preferentially labeled by lactoperoxidase-catalyzed radioiodination (D. Hoessli, unpublished observations).

### *Labeling Procedures*

Purified pig skeletal muscle α-actinin (30 μg) was iodinated with 5 μg of lactoperoxidase (Sigma), 5 μl of a 0.04 μM solution of $H_2O_2$ diluted in a 5 mM $PO_4$, pH 7.4, 5 mM $MgCl_2$ buffer and 300 μCi of carrier-free $^{125}I$ (The Radiochemical Center, Amersham, England). The reaction was carried out at 37°C for 30 min and stopped by adding 4 vol of ice-cold PBS. Macromolecular iodinated material was collected in the void volume of a G-25 column. Variable amounts of lactoperoxidase were self-iodinated using this procedure, but did not overlap with α-actinin in 2-D gel electrophoresis.

Biosynthetic labeling of spleen lymphocytes was carried out in methionine-free Dulbecco's modified Eagle's medium (DMEM), supplemented with L-glutamine (2 mM), antibiotics, and 10% dialyzed foetal calf serum (FCS). 2-ml cultures were set

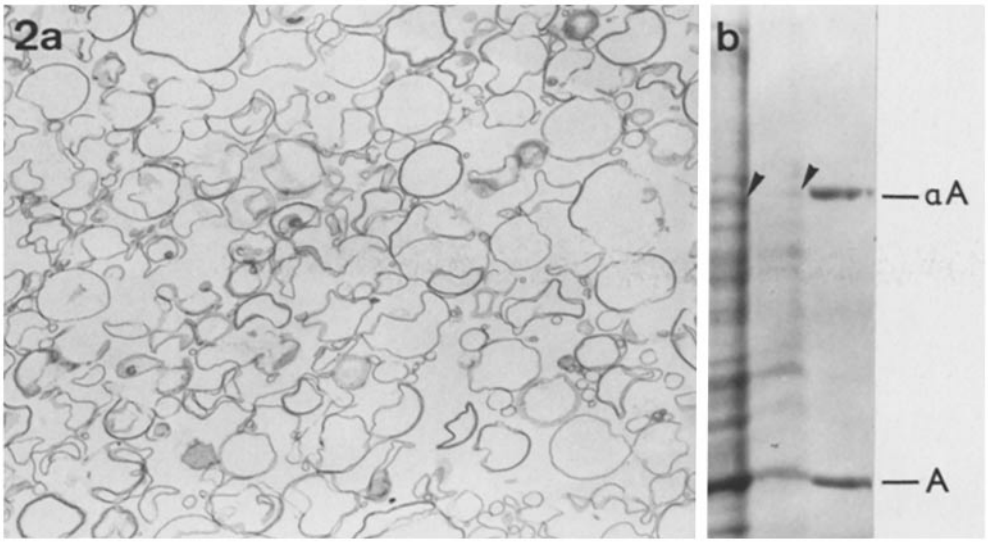


FIGURE 2 Characterization of purified membranes from P1798 lymphoma cells. (*a*) Electron micrograph of the top fraction (collected between the overlaying buffer and 22.5% sucrose). × 8,100. (*b*) 7.5% Polyacrylamide gel stained with Coomassie blue. Left lane: floated fraction between 35% and 22.5% sucrose. Middle lane: floated fraction between 22.5% sucrose and overlaying buffer. Right lane: skeletal muscle α-actinin (αA) (10 μg), skeletal muscle actin (*A*) (10 μg) used as markers. A band comigrating with α-actinin and a band comigrating with actin are visible in both membrane fractions.

up containing a total of $20 \times 10^6$ cells. 200 μCi of [$^{35}$S]methionine (obtained locally by labeling *Escherichia coli* with $^{35}SO_4$, hydrolyzing the labeled products in HCl and separating the $^{35}$S containing cysteine and methionine on Dowex 50) were added to each culture. After 4 h of incubation at 37°C in a humidified 5% $CO_2$ atmosphere, the cells were washed three times in HBSS and lysed in PBS-Nonidet P-40 0.5% (vol/vol) (PBS-NP-40), 1 mM phenyl-methyl-sulfonyl-fluoride (PMSF). The lysates were centrifuged at low speed to remove nuclei and clarified at 100,000 *g* for 30 min immediately before immunoprecipitation.

Cell surface iodination: $40–60 \times 10^6$ cells (>95% viable) were incubated in PBS-glucose 20 mM with 20 μg of lactoperoxidase (Sigma), 2 U of glucose-oxidase (type V, Sigma) and 1 μCi of carrier-free $^{125}$I (Amersham Radiochemical Centre) for 40 min at 20°C. After three washes in cold PBS, cells were lysed in PBS-NP-40 0.5%, 1 mM PMSF, for 30 min at 20°C. Nuclei were sedimented at 3,000 *g* for 15 min and the supernate was passed over G-25 Sephadex in PBS-NP-40 0.5% to remove unbound iodine. Material in the excluded volume was pooled, centrifuged at 100,000 *g* for 30 min, and used for immunoprecipitation.

## *Immunoprecipitation*

Aliquots of lysate corresponding to $5–8 \times 10^6$ cells were submitted to immunoprecipitation. 50–80 μg of anti-α-actinin antibody, 5–10 μg of anti-membrane Ig fraction or anti-surface antibody were incubated for 30 min at 37°C with the lysate. To select Con A binding biosynthetic products, 5 μg of Con A (type V, Sigma) were added first, and the mixture was kept at 37°C for 30 min. Affinity-purified anti-Con A antibody was then added (10 μg) for a further 30 min of incubation under the same conditions. As a control, lysates were incubated with the anti-Con A antibody without preincubation with the lectin. Immune complexes were adsorbed onto heat-killed, formaldehyde-treated Cowan I strain *Staphylococcus aureus* (20 μl of a 10% vol/vol suspension) and incubated for 20 min at 20°C. Bacterial pellets were then handled as described by Kessler (27). The radioactivity bound to the adsorbent was eluted by resuspending the bacteria in 9.5 M urea, 2% ampholines, 2% NP-40, 5% β-2 mercaptoethanol (β-2 ME). Over 75% of the radioactivity appeared in the supernate after 15 min of incubation at 20°C. Alternatively, aliquots of lysate were directly mixed with the above buffer, without prior immunoprecipitation.

## *2-D Polyacrylamide Gel Electrophoresis*

The 2-D gel electrophoresis method developed by O'Farrell (40) was followed with minor modifications. Isoelectric focusing gels were collected into SDS sample buffer and frozen at −70°C. Before loading on the second dimension, the gels were equilibrated in three changes of SDS sample buffer at 20°C for 10 min each. The second dimension was carried out on 7.5% polyacrylamide slab gels. The pH gradient extended from 4.5 to 7.5, with a value of 6.4 in the middle. Since these pH values were measured in the presence of urea, they do not represent exact values (40); pH values are given in the text and figure legends, only to localize the segment shown in the pH gradient.

The following materials were used: sodium dodecyl-sulfate (SDS) (Serva, Heidelberg, Germany); urea, ultra-pure grade (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.); ampholines (LKB, Stockholm, Sweden); acrylamide and *N,N'*-methylene-bisacrylamide (Serva, Heidelberg, Germany); *N,N,N',N'*-tetramethylenediamine (TEMED) (Fluka, Buchs, Switzerland).

Autoradiography: the 2nd dimension slab gels containing [$^{35}$S]methionine were impregnated with 2.5-diphenyloxazole, according to Bonner and Laskey (4). Dried gels were exposed to Kodak RP Royal X-Omat films at −70°C. Exposure times varied between 5 and 15 d. $^{125}$I-containing slab gels were dried immediately after electrophoresis and exposed to Kodirex (Kodak, Lausanne, Switzerland) film at room temperature for 10–20 d.

## *Immunofluorescence Staining*

Spleen lymphocytes were isolated from DBA/2 mice, and $60 \times 10^6$ cells were incubated at 0°C in 0.5 ml of HBSS to which an equal amount of rhodamine-conjugated goat antiserum against mouse immunoglobulins (GAMIG) (8, 31) (Nordic Co., Lausanne, Switzerland) was added. T-lymphocytes were isolated from peripheral lymph nodes. In some experiments, T-cell enrichment was achieved by nylon wool column filtration (26).

$60 \times 10^6$ lymph node cells were incubated for 30 min at 0°C with 0.3 ml of HBSS to which 0.1 ml of an anti-Thy-1.2 alloantiserum (43) was added. For the determination of anti-Thy-1 independent capping, aliquots were incubated with normal mouse serum as a control. After three washes in HBSS, peripheral lymphocytes were incubated for 30 min at 0°C with fluorescein-conjugated GAMIG diluted ½ in HBSS.

From the suspensions of spleen lymphocytes or peripheral cells, aliquots of $15 \times 10^6$ cells were prepared; 1 aliquot was immediately fixed at room temperature by adding an equal amount of 0.2% paraformaldehyde in PBS. The other aliquots were placed at 37°C, and capping was stopped by adding equal amounts of 0.2% paraformaldehyde at room temperature, at the following intervals: 5, 10, and 30 min. After three washes in PBS, drops of the suspensions were applied on slides and air dried. Dried cells were fixed for 30 s in absolute ethanol, incubated for 15 min with purified rabbit anti-α-actinin antibody (50 μl, 0.5 mg/ml) and, for a further 15 min, with a fluorescein- (splenocytes) or rhodamine- (peripheral lymphocytes) conjugated Ig fraction of a goat antiserum against rabbit IgG (GARIG) (Miles Seravac, Lausanne, Switzerland, or Behring Werke, Marburg Lahn, West Germany). As a control, rabbit γ-globulins (50 μl, 0.5 mg/ml, Fluka) were used instead of anti-α-actinin antibody. To avoid cross-reactions among antisera, GAMIG-rhodamine or -fluorescein was absorbed on a solid immunoabsorbent of glutaraldehyde-insolubilized rabbit Ig; mouse anti-Thy-1 was absorbed on rabbit Ig and goat Ig; GARIG-fluorescein and -rhodamine were absorbed on mouse Ig; purified anti-α-actinin antibodies and normal rabbit immunoglobulins were absorbed on mouse Ig and goat Ig.

Photographs were taken with a Zeiss ultra-violet photomicroscope equipped with epi-illumination and specific filters for rhodamine and fluorescein, using a plan Apo-chromate × 63/1.0 objective on HP5 Ilford black-and-white film or EKTA 400 Kodak color film.

# RESULTS

## *Identification and Characterization of α-Actinin by 2-D Gel Electrophoresis*

In an attempt to localize α-actinin on the 2-D map of biosynthetically labeled mouse lymphocyte proteins, iodinated pig skeletal muscle α-actinin was mixed with a lysate of [$^{35}$S]methionine-labeled spleen lymphocytes.

Fig. 3 shows 2-D maps of the spleen lymphocyte

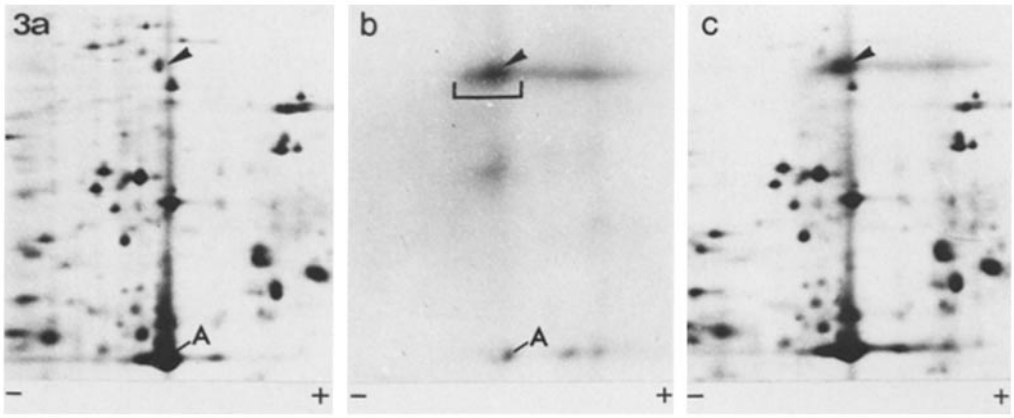


FIGURE 3 Identification by 2-D gel electrophoresis of a DBA mouse spleen lymphocyte protein comigrating with pig skeletal muscle α-actinin. (*a*) 200,000 cpm of a [$^{35}$S]methionine labeled lysate of lymphocytes corresponding to $1 \times 10^6$ cells. (*b*) $^{125}$I-Labeled pig skeletal muscle α-actinin (200,000 cpm in 300 ng of protein). The bracket indicates the mapping position of the cold α-actinin. (*c*) Aliquots of samples described in *a* and *b* mixed before isoelectric focusing. Arrowheads point to the iodinated muscle α-actinin (*b* and *c*) and to the biosynthetically labeled lymphocyte protein (*a*). Estimated mol wt for skeletal muscle α-actinin: 100,000. *A* designates actin (mol wt: 42,000) in the lysate (*a*) as well as in the marker preparation (*b*). The portion of the pH gradient extending from pH 6.8 (−) to pH 5.6 (+) is shown.

lysate (*a*), the marker (*b*), and the mixture of the two materials (*c*). The bulk of the iodinated marker (Fig. 3 *b*, bracket) maps with the same coordinates as the cold α-actinin (Fig. 1 *a*). Additional radioactivity, however, extends from the main spot towards the acid end. Since no such trailing is observed with the cold protein, this probably represents negative charges introduced by iodination. The 1% actin contamination of the α-actinin marker which is not apparent by Coomassie blue staining (Fig. 1 *a*) is enhanced by iodination, since actin contains more tyrosine that α-actinin. The two spots to the right of actin are probably due to iodination artifacts. On the other hand, the spot of ~80,000 mol wt is a breakdown product of α-actinin found after iodination with lactoperoxidase (B. M. Jockusch, unpublished observations). The $^{125}$I track of the α-actinin marker is distinguishable among the [$^{35}$S]methionine spots of the lymphocyte lysate (Fig. 3 *c*). The marker can be localized as the middle component of a group of three spots. A similar group of spots is apparent on the map of the spleen lymphocyte lysate (Fig. 3 *a*) and a well-labeled [$^{35}$S]methionine spot occupies the middle position within this group. Hence, a well-defined [$^{35}$S]methionine-labeled spot that comigrates with skeletal muscle α-actinin can be defined in a spleen lymphocyte lysate.

To further characterize this mouse lymphocyte protein with an electrophoretic mobility and isoelectric point identical to that of skeletal muscle α-actinin, the same internally labeled lymphocyte lysate was subjected to immunoprecipitation with anti-α-actinin antibody. A labeled spot with the expected α-actinin position is detectable in the unselected lysate (Fig. 4 *a*, arrow). The anti-α-actinin immunoprecipitate contains an identically located spot (Fig. 4 *b*, arrow), which is strongly enriched as compared to its relative intensity in the unselected lysate. By contrast, contaminating spots in this specific immunoprecipitate are much less intense than in the total lysate. The presence of actin (identified by comigration with skeletal muscle actin) in immunoprecipitates is a common finding (24, 25). This probably reflects nonspecific binding to immunoglobulins in solutions, since anti-α-actinin antibody does not label actin by immunoperoxidase staining (Fig. 1 *b*).

The possible glycoprotein nature of the immunoprecipitated protein was investigated by incubating the lymphocyte lysate with Con A. The lectin-binding glycoproteins were then collected with an anti-Con A antibody. Fig. 4 *c* shows that Con A-binding glycoproteins do not include a spot with the expected α-actinin location. Therefore, mouse lymphocytes containing a protein which comigrates with skeletal muscle α-actinin, is precipitated by antibodies against skeletal muscle α-actinin, and is not a Con A-binding glycoprotein.

The relationship of lymphocyte α-actinin with

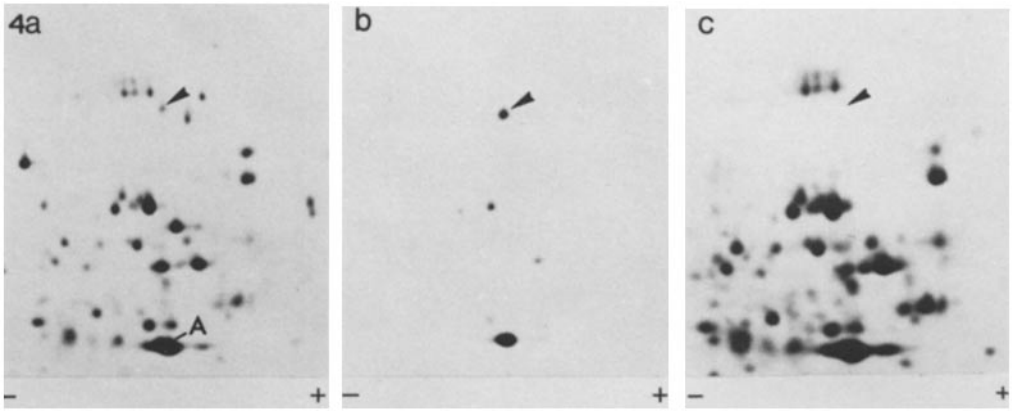


FIGURE 4 Immunoprecipitation of a [$^{35}$S]methionine-labeled DBA spleen lymphocyte lysate with α-actinin or Con A plus anti-Con A antibodies. 2-D analysis of unselected lymphocyte lysate and of immunoprecipitates. (*a*) 200,000 cpm of a [$^{35}$S]methionine labeled, DBA spleen lymphocyte lysate corresponding to $1 \times 10^6$ cells. (*b* and *c*) $1.6 \times 10^6$ cpm of the same lysate immunoprecipitated with 50 μg of anti-α-actinin antibody (*b*) or incubated with 5 μg of Con A and immunoprecipitated with 10 μg of anti-Con A antibody (*c*). Arrowheads, *A*, (+), and (−) as in Fig. 3.

cell membranes was studied by incubating the lymphocyte lysate with antibodies directed against either the whole membranes or the external surface of mouse lymphocytes. After immunoprecipitation with the anti-membrane antiserum, a spot at the position of α-actinin was identified (Fig. 5 *c*). An identical spot was detectable in the maps of both unselected lysate (Fig. 5 *a*) and anti-α-actinin immunoprecipitate (Fig. 5 *b*). However, precipitation with the anti-surface antibody did not result in the detection of this spot (Fig. 5 *d*). In addition to the 40,000–50,000 mol wt spots immunoprecipitated with the anti-surface antibody, major glycoproteins of 150,000 and 200,000 mol wt are also detected (D. Hoessli, unpublished observations), but are not included in the portion of the map shown in Fig. 5 *d*. Lactoperoxidase-catalyzed surface labeling of spleen lymphocytes with $^{125}$I was ineffective in demonstrating a spot in the position of α-actinin (Fig. 5 *e*). Moreover, immunoprecipitation of the surface-labeled cell lysate with anti-α-actinin antibody or anti-membrane antiserum did not detect any α-actinin spot (data not shown).

### *Double Immunofluorescence with Anti-Ig, Anti-Thy-1, and Anti-α-actinin Antibodies*

After incubation in suspension with GAMIG-rhodamine, 70% of spleen lymphocytes stained positively (Fig. 6 *a–c*). At 0°C, the cells were diffusely labeled along the cell membrane with some patching and capping. Cells incubated at 37°C showed progressively increasing redistribution of surface Ig into caps (Fig. 6 *d–f*). We counted 62% capped spleen lymphocytes at 5 min, 78% at 10 min, and 90% at 30 min (Fig. 7 *a*). Approximately 85% of these Ig caps showed a co-cap of α-actinin at all time points (Fig. 7 *a*). Noncapped cells had practically no α-actinin fluorescence over the nuclear area and a weak fluorescence at the cell periphery; in Ig-capped cells, the fluorescence for α-actinin was clearly visible and restricted to the cap (Fig. 6 *d–f*). In general, the intensity of the immunofluorescent staining for α-actinin was weaker than the staining for actin and tubulin under similar conditions (14).

Lymph node lymphocytes incubated with anti-Thy-1 at 0°C showed diffuse, weak membrane-associated staining. Upon warming to 37°C, the diffusely distributed fluorescence progressively accumulated in typical caps (Fig. 8 *a–c*). Thus, we counted 42% capped cells at 5 min, 56% at 10 min, and 60% at 30 min (Fig. 7 *b*). The caps of the Thy-1 antigen were larger than Ig caps of B cells. At all time points, virtually all staining with anti-α-actinin antibodies was associated with the Thy-1 cap area. Fig. 7 *b* shows that ~20% of peripheral lymph node lymphocytes capped after incubation with normal mouse serum instead of anti-Thy-1 antiserum. This proportion reflects the expected number of B cells in such a cell population.

## DISCUSSION

We have shown that mouse lymphocytes synthe-

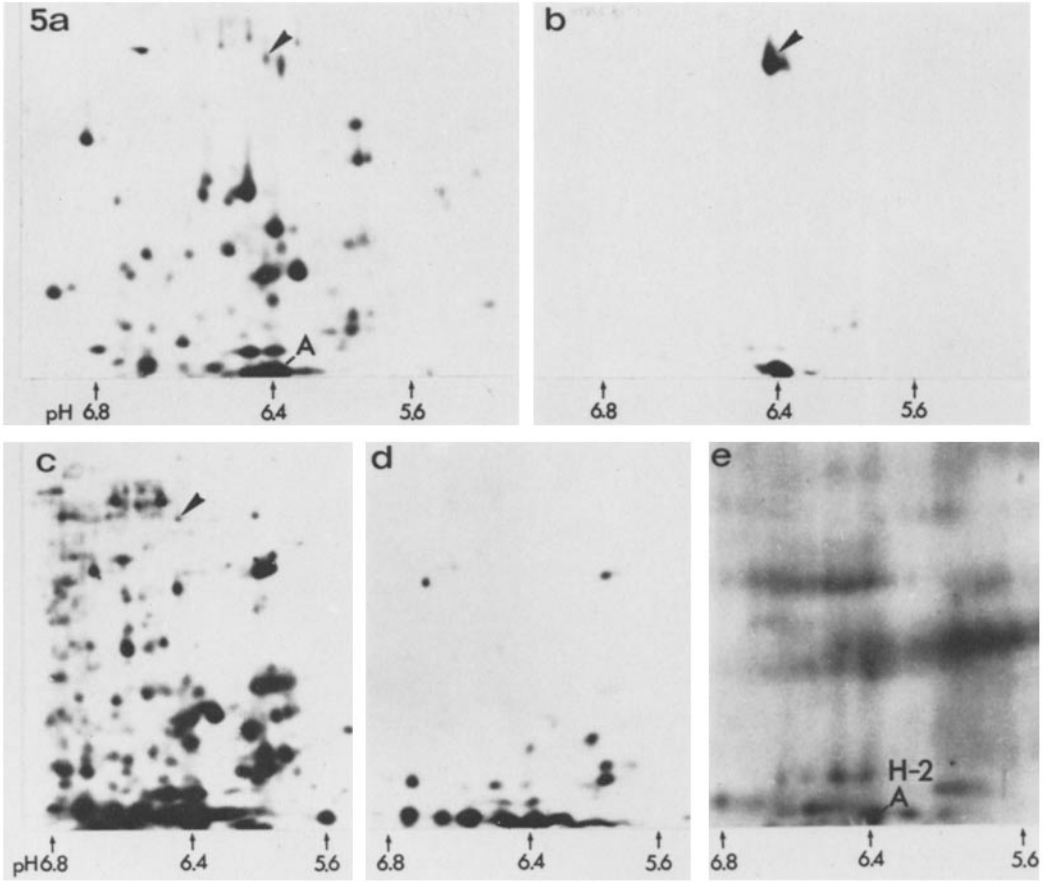


FIGURE 5 Relationships between biosynthetically labeled α-actinin and the cell membrane. 2-D analysis of the unselected cell lysate and anti-α-actinin, anti-membrane and anti-surface immunoprecipitates (*a–d*) and of surface radioiodinated products (*e*). (*a*) 200,000 cpm of a [$^{35}$S]methionine-labeled, DBA spleen lymphocyte lysate corresponding to $1 \times 10^6$ cells. (*b–d*) $1.5 \times 10^6$ cpm of the same lysate immunoprecipitated with 80 μg of anti-α-actinin antibody (*b*), 10 μg of anti-membrane immunoglobulin fraction (*c*), or 5 μg of anti-surface antibody (*d*). (*e*) 250,000 cpm of a $^{125}$I surface-labeled, DBA spleen lymphocyte lysate corresponding to $2 \times 10^6$ cells. The position of H-2 has been determined by comparison with the H-2 maps of the same material published by Jones (24). Arrowheads and *A* as in Fig. 3. (+) and (−) describe a portion of the pH gradient ranging from ~6.8 to 5.6.

size a protein, which, in a 2-D polyacrylamide gel electrophoresis system, comigrates with α-actinin from pig skeletal muscle. This lymphocyte protein can be specifically immunoprecipitated with an antibody directed against skeletal muscle α-actinin which itself only binds to α-actinin and its proteolytic cleavage products as shown by immunoreplica staining. The fact that actin is not stained by this method shows that no anti-actin antibody is contained in our antiserum. The presence of actin in immunoprecipitates prepared in nonionic detergent lysates could be due to either nonspecific binding of actin to immune complexes (24) or to specific interaction of actin with the antigen when the antigen is a cytoskeletal protein such as α-actinin. Hence, it appears that mouse lymphocytes synthesize a protein which is antigenically similar to the α-actinin present in Z-lines of striated muscle fibers (19, 34). Although α-actinin is reproducibly labeled with [$^{35}$S]methionine, it represents a relatively minor radioactive spot as compared to the intensity of the actin spot on the same map. However, since muscle actin contains 1.6 times more methionine when compared to α-actinin (12,

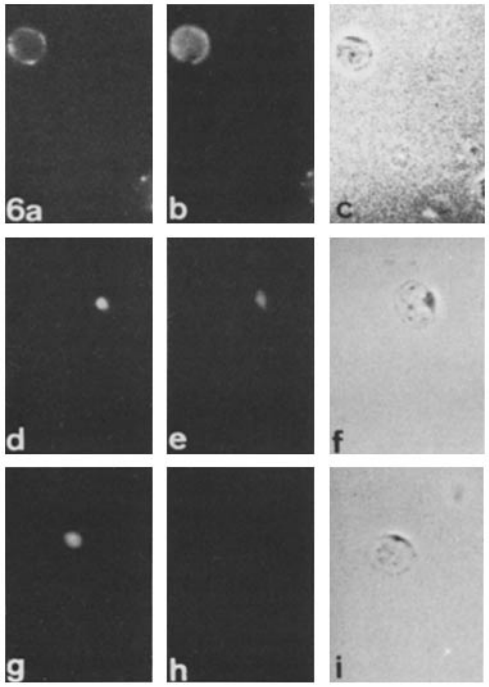


FIGURE 6 Immunofluorescent staining of splenic B lymphocytes incubated in suspension with GAMIG-rhodamine, then fixed in ethanol and incubated with anti-α-actinin antibody followed by anti-rabbit IgG-fluorescein. (*a–c*) Incubation in suspension at 0°C; *a*, rhodamine; *b*, fluorescein; *c*, phase contrast. The α-actinin staining is distributed at the periphery of the lymphocytes similarly to staining with GAMIG. (*d–f*) Incubation in suspension at 37°C; *d*, rhodamine; *e*, fluorescein; *f*, phase contrast. Here, Ig show a typical cap formation and α-actinin staining is concentrated beneath the cap. (*g–i*) Incubation in suspension at 37°C; as control, after ethanol fixation, cells were incubated with rabbit Ig instead of anti-α-actinin antibody and then with anti-rabbit IgG. *g*, rhodamine; *h*, fluorescein; *i*, phase contrast. No α-actinin staining is present beneath the Ig cap. × 2,000.

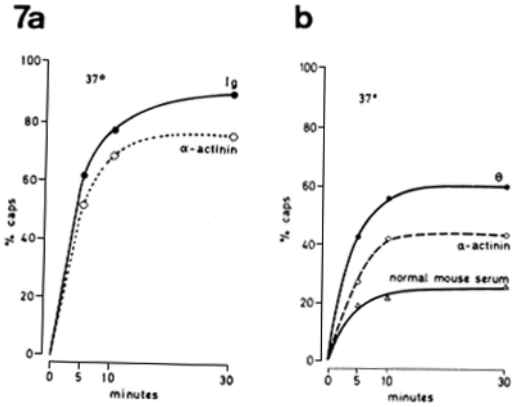


FIGURE 7 Capping kinetics of Ig and Thy-1 at 37°C and concurrent redistribution of α-actinin. (*a*) Mouse spleen lymphocytes were incubated with GAMIG-rhodamine at 0°C and aliquots were then placed at 37°C for 5, 10, and 30 min. (*b*) Mouse peripheral lymph node lymphocytes were incubated with anti-Thy-1 (*θ*) or normal mouse serum at 0°C, washed, and then incubated with GAMIG-fluorescein. The cells were placed at 37°C for 5, 10, and 30 min. In all cases, capping was stopped by fixation with 0.2% paraformaldehyde. Washed cells were placed on a slide and incubated with anti-α-actinin antibody, followed by GARIG-fluorescein for spleen cells and GARIG-rhodamine for lymph node cells. (*% caps*) Ratio of capped cells to surface stained cells.

44), labeling lymphocytes with [$^{35}$S]methionine may not reflect the true proportion of these two proteins in nonmuscle cells.

It was of interest to assess the carbohydrate content of this molecule, in view of the recently postulated identity of α-actinin and glycoprotein III in platelets (16). With the lectin Con A as a ligand, the mannose-containing glycoproteins can be selected (17), which are characterized by simple or complex carbohydrate chains linked through *N*-glycosidic bonds to asparagine residues in the polypeptide chain (29). α-Actinin did not bind to Con A in our system and probably does not belong to this category of glycoproteins.

The ability of the anti-membrane antiserum to immunoprecipitate α-actinin is consistent with the presence of a protein comigrating with α-actinin in purified membrane preparations. α-Actinin (38) as well as actin (3) have already been shown to copurify with membrane fractions. Moreover, freeze-etching analysis of thymocyte membranes prepared by the technique used in our studies revealed the presence of filament bundles attached to the lipid bilayer (36). These filamentous structures could be likely sources of cytoskeletal proteins in membrane preparations. Trapping of cytoplasmic material in sealed vesicles cannot be excluded but seems a less likely explanation in view of the electron microscope homogeneity of such membrane preparations.

Since the incubation of this anti-membrane antiserum with fixed cells did not result in the adsorption of anti-α-actinin antibodies, we conclude that α-actinin antigenic determinants are not accessible at the cell surface. This, taken together with the failure of α-actinin to bind Con A and to become labeled by lactoperoxidase-catalyzed ra-

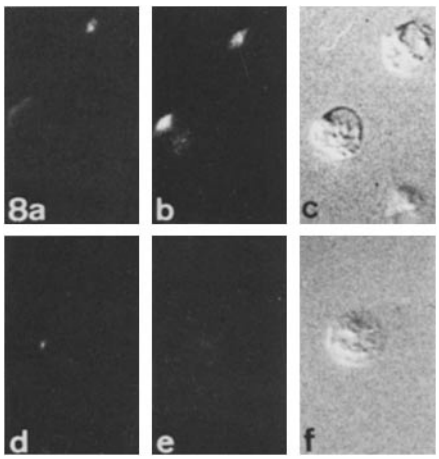


FIGURE 8 Immunofluorescent staining of T lymphocytes incubated in suspension with anti-Thy-1 and GAMIG-fluorescein, then fixed with ethanol and incubated with anti-α-actinin antibody followed by anti-rabbit IgG-rhodamine. (*a–c*) Incubation in suspension at 37°C; *a*, fluorescein; *b*, rhodamine; *c*, Nomarski differential interference microscopy. α-Actinin staining is localized beneath the caps of Thy-1 antigens. (*d–f*) Incubation in suspension at 37°C; as control, after ethanol fixation, cells were incubated with rabbit Ig instead of anti-α-actinin antibody, and then with anti-rabbit IgG; *d*, fluorescein; *e*, rhodamine; *f*, Nomarski differential interference microscopy. No staining corresponds to the cap formation of Thy-1 antigens. × 2,000.

dioiodination, suggests that in lymphocytes the α-actinin molecule of 100,000 mol wt recognized by the antibody made against the muscle protein is an internal protein. However, our data do not exclude the possibility that intact lymphocytes may contain a larger α-actinin molecule, whose additional portion could be an integral membrane protein with carbohydrates attached to its external protrusion. Evidence for the existence of such a protein has been presented (21). This additional portion could be dissociated from the 100,000 mol wt α-actinin molecule during non-ionic detergent solubilization of the whole cells and would therefore escape detection by immunoprecipitation with anti-α-actinin antibody.

The association of α-actinin with the plasma membrane has been proposed in several experimental models, such as attachment sites of fibroblasts to the substrate (45), dense bodies of smooth muscle (46), the terminal web of intestinal epithelial cells (7, 9), synaptic vesicles (42, 48) and secretory vesicles of platelets and adrenal medulla cells (22). Based on this indirect body of evidence, a model has been proposed which includes α-actinin in the architecture of the lymphocyte plasma membrane (35).

The dynamic aspect of the association of α-actinin with the plasma membrane is illustrated by the intracellular redistribution of this protein after ligand-induced patching and/or capping of surface glycoproteins. Our kinetic studies indicate that, up to 30 min, α-actinin remains stably associated with Ig and Thy-1 antigen aggregates. A recent study (6) has shown that myosin, on the contrary, is associated only at 5 min with capped Ig and is never significantly associated with Thy-1. α-Actinin has also been shown to co-cap with other lymphocyte surface antigens (15); hence the association of α-actinin with aggregated surface components is probably a general phenomenon. It appears that such an association is more stable than that of myosin under similar conditions.

α-Actinin has been shown both in vitro (18, 47) and in vivo (32, 33) to interact with actin by orienting the latter protein to permit efficient contraction of the actomyosin complex. We present evidence that an α-actinin molecule similar to the Z-line protein is indeed synthesized by lymphocytes. This chemical analogy also suggests a structural and functional analogy between α-actinin in lymphocytes and that in muscle cells. The demonstration that α-actinin co-caps with surface Ig and Thy-1 antigen suggests that α-actinin is involved in the movement of surface receptors in lymphocytes.

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