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STRUCTURAL ANALYSIS OF THE MYELOMA-ASSOCIATED MEMBRANE ANTIGEN KMA

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κ-Myeloma antigen (KMA) was immunoprecipitated from lactoperoxidase-radioiodinated HMy2 lymphoblastoid cells by using monoclonal antibody K-1-21 and was analyzed by SDS-PAGE. Under reducing conditions, two major subunits of Mr ≈ 26,000 and Mr ≈ 42,000, and minor components of Mr ≈ 28,000, 31,000, and 36,000 were observed. The Mr ≈ 26,000 subunit was identical to κ-light chains from HMy2 surface IgG in apparent m.w., isoelectric point, and staphylococcal V-8 protease peptide map, but was not precipitated in association with Ig heavy chain. The Mr ≈ 42,000 component was homologous to rabbit skeletal muscle actin by peptide mapping with staphylococcal V-8 protease. The cell surface origin of the immunoprecipitated antigen was confirmed by demonstrating lactoperoxidase dependence of iodination and complete removal from the cell surface after pronase treatment of viable cells. Thus, cell surface expression of KMA is the result of membrane association of non-heavy chain-linked κ-light chains, possibly in noncovalent association with actin.

The monoclonal antibody K-1-21, with specificity for free but not heavy chain-associated human κ-light chains, recognizes a cell surface antigen present on tumor cells from patients with κ-myeloma, κ-Waldenstrom's macroglobulinemia, and κ-non-Hodgkin's lymphoma. The antigen (κ-myeloma antigen; KMA)[3] is not detected on cells from non-neoplastic adult lymphoid tissues (1), but may be expressed on some fetal B cells and on normal adult B cells stimulated in vitro (manuscript in preparation).

We have sought to explain this observed pattern of expression by structural characterization of KMA. From previous results, the correlation between light chain isotype expression and KMA expression among patients with myeloma and non-Hodgkin's lymphoma indicates that KMA is probably a product of the κ-locus. By using the human lymphoblastoid cell line LICR-LON HMy2, which expresses KMA and intact IgG (1, 2), we have demonstrated the heavy chain independence of KMA by capping studies (3). KMA is capped on cells treated with K-1-21 plus sheep anti-mouse Ig, conditions which fail to cap surface IgG. Furthermore, KMA can be specifically capped by polyvalent antisera to human κ-light chains but not by antisera to Ig heavy chains, suggesting that the antigen consists structurally of free κ-light chains stably associated with the plasma membrane (3). In this paper, we confirm by immunoprecipitation of labeled cell surface proteins that KMA is indeed composed of free κ-light chains. In addition, we note that a number of other polypeptides, and in particular actin, are noncovalently associated with this antigen. The association with actin may be the means by which free light chains are expressed on the cell surface as KMA.

MATERIALS AND METHODS

Antibodies. K-1-21, an IgG1, κ monoclonal antibody specific for free human κ-light chains described previously (1, 2), was affinity-purified from ascites fluid by using a κ-light chain affinity column. F-1-1, an IgG1, κ monoclonal antibody of irrelevant specificity, was purified from ascites fluid by affinity chromatography on protein A (4). WESTMEAD-2 (WM-2) is an IgG2a, κ monoclonal antibody specific for a monomorphic determinant of HLA-DR, and was the generous gift of Dr. K. Bradstock (Department of Medicine, Westmead Hospital, NSW).

Cell lines. The line LICR LON/HMy2, derived from a patient with plasma cell leukemia (2), is an IgG κ-synthesizing B lymphoblastoid cell line obtained from the Ludwig Institute of Cancer Research, London, UK. The line MW-28 is an IgG, κ-synthesizing B lymphoblastoid cell line produced by in vitro transformation with EBV, and was the gift of Dr. J. Gibson (University of Sydney). All cell lines were grown as described (1).

Radiolabeling and lysis of cells. Cells were washed twice in cold PBS, pH 7.2, and 2×10^7 cells were resuspended in 1 ml PBS. The cells were radiolabeled according to the method of Meuer et al. (5) by the addition of 0.5 M D-glucose (10 μl), 0.5 mM $K^{127}I$ (5 μl), 2 mg/ml lactoperoxidase (Sigma Chemical Co., St. Louis, MO) (10 μl), 1 mCi carrier-free Na ^{125}I (Amersham, Bucks, UK), and 7.5 U/ml glucose oxidase (Sigma) (20 μl). After incubation for 20 min at room temperature, 50 μl 0.1 M KI were added, incubation was continued for an additional 2 min, and the cells washed three times in cold RPMI + 0.02% NaN_3. Viability was determined immediately before and after radiolabeling by staining with acridine orange and ethidium bromide (6).

Labeled cells were solubilized in 2 ml lysis buffer (1% NP-40, 10 mg/ml BSA, 900 KIU/ml aprotinin, 1 mM PMSF, 1 mM EGTA, 5 mM KI, 0.02% NaN_3 in PBS, pH 7.2) for 30 min on ice. Nuclei and detergent-insoluble material were removed by centrifugation at 3500 × G for 10 min at 4°C.

Immunoprecipitation of KMA. Immunoprecipitation of ^{125}I-labeled cell surface proteins was carried out by using a modification of the method of Gallatin et al. (7). K-1-21, F-1-1, WM-2, or protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) were bound to the wells of polyvinyl chloride (PVC) round-bottomed microtiter trays (Cooke Laboratories, Dynatech Corp., Alexandria, VA) at 100 μg/ml in PBS/0.02% NaN_3. The wells were washed twice in PBS containing 0.05% Tween 20 (Sigma Chemical Co.), once in PBS alone, were blocked by a 1-hr incubation with BSA (10 mg/ml in PBS), and were washed in PBS-Tween followed by PBS alone. Antigen blocking of K-1-21 was used to assess nonspecific binding of labeled proteins in this system. Control wells (usually 12 to 16 per experiment) coated with K-1-21 were incubated for 2 hr at 37°C with κ-BJP (1 mg/ml) while λ-BJP (1 mg/ml) was added to the test wells. The trays were

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[1] Supported by a grant from the University of Sydney Cancer Research Committee.
[2] To whom correspondence should be addressed.
[3] Abbreviations used in this paper: KMA, κ-myeloma antigen; IEF, isoelectric focusing; HGG, human γ-globulin; BJP, Bence Jones Protein.

washed as described, and 50-μl aliquots of ^{125}I-labeled HMy2 cell lysates containing κ-BJP or λ-BJP at final concentrations of 1 mg/ml were added to the control and test wells, respectively. Aliquots of HMy2 lysates were also added to F-1-1, WM-2, and protein A-coated wells. After a 16-hr incubation at 4°C, the wells were washed five times in 2% NP-40, 1 mM EGTA, 10 mM Tris, pH 7.2, three times in PBS-Tween, and twice in PBS alone. Bound proteins were then eluted by the addition of 100 μl 0.05 M triethylamine, pH 11.6, to each well. After incubation at room temperature for 30 min, aliquots from wells within each treatment group were pooled separately and were lyophilized immediately.

Preabsorption of lysates. Lysates (500-μl aliquots) of ^{125}I-labeled HMy2 cells were mixed with 200 μl packed protein A-Sepharose CL-4B (Pharmacia) or human γ-globulin (HGG) (Commonwealth Serum Laboratories, Melbourne, Australia) coupled to CNBr Sepharose 4B, and were incubated for 2 hr at 4°C before centrifugation at 1000 × G for 5 min.

One-dimensional polyacrylamide gel electrophoresis (PAGE). Lyophilized samples were dissolved in 25 to 50 μl of SDS sample buffer (7) containing 4 mg/ml dithiothreitol (DTT), were boiled for 3 min, and were electrophoresed according to the method of Laemmli (8). Gels were stained with Coomassie Blue to visualize m.w. standards (Pharmacia), then were dried and were autoradiographed for 1 to 5 days at −70°C with Kodak X-AR-5 film (Kodak Australasia Pty. Ltd., Sydney, Australia) and Dupont lighting plus intensifying screens (E. I. Dupont de Nemours and Co., Wilmington, DE).

Isoelectric focusing (IEF). IEF was performed in 0.5-mm-thick horizontal slab gels containing 5% acrylamide, 1.6% pH 3 to 10 ampholytes (Pharmacia), 2% NP-40, and 6.9 M urea. Anodal and cathodal wicks were soaked in 1 M NaH_3PO_4 and 1 M NaOH, respectively. Electrofocusing was carried out for 3000 Volt hours at 10°C. The gels were fixed, were stained with Coomassie Blue, were dried, and autoradiography was performed as described for SDS-PAGE.

Two-dimensional separations. Two-dimensional (size, charge) separation was performed essentially according to the method of Sidman (9). Strips excised from dried 10% acrylamide SDS gels were rehydrated in IEF sample buffer (1.7% pH 3 to 10 ampholytes, 2% NP-40, 6.9 M urea, and 5% v/v 2-mercaptoethanol) for 1 hr. The rehydrated strips were applied to the surface of the slab IEF gel, and were removed at 1500 Volt hours, after which time electrofocusing was continued for an additional 1500 Volt hours. For two-dimensional (charge, size) analysis, lyophilized samples were dissolved in IEF sample buffer and were electrofocused on a horizontal slab gel in the first dimension as described above. Immediately after completion of focusing, strips 7.5 mm wide were excised, were equilibriated 5 min in SDS sample buffer, and were sealed into the top of a second dimension Laemmli gel with 1% agarose in SDS sample buffer. Electrophoresis, staining, and autoradiography were performed as described above.

Peptide mapping. Polypeptide-containing strips were excised from dried 10% acrylamide SDS gels and were rehydrated for 1 hr in SDS sample buffer containing 4 mg/ml DTT. The strips were placed in sample wells, were overlayed with 40 μl staphylococcal V8-protease (Sigma) in SDS sample buffer, and were electrophoresed according to the method of Cleveland et al. (10), but with the inclusion of 4.5 M urea in the stacking gel as suggested by Handman et al. (11). Purified rabbit skeletal muscle actin (the generous gift of Dr. J. Barden, University of Sydney) and purified κ-Bence Jones Proteins (BJP) were digested in a similar fashion, with the exception that they were in solution and were layered in the bottom of sample wells in SDS sample buffer with 20% glycerol and were then overlayed with protease solution.

Pronase treatment of HMy2 cells. ^{125}I-labeled HMy2 cells were resuspended at a density of 2×10^6 cells/ml in RPMI 1640 with 1 mg/ml pronase (Calbiochem, San Diego, CA) and were incubated at 37°C for 15 min. The cells were then diluted in RPMI with 10% FCS and were washed twice in RPMI/FCS and twice in RPMI alone. Control cells were treated identically, but with the omission of pronase from the solution.

RESULTS

Immunoprecipitation of KMA. The plasma cell leukemia-derived B lymphoblastoid cell line LICR LON/HMy2 was chosen for this study because the line expresses KMA on approximately 85% of cells (1) at a mean density of 10^4 molecules/cell as determined by Scatchard plot analysis using ^{125}I-labeled K-1-21 (data not shown). Cells were radioiodinated, were solubilized in NP-40, and KMA was immunoprecipitated by using K-1-21 bound to PVC microtiter wells in the presence of excess κ- (control) or λ- (test) light chains. As additional specificity controls, immunoprecipitation was also performed in wells coated with an irrelevant IgG1-κ monoclonal antibody (F-1-1), and a monoclonal antibody to HLA-DR (WM-2). When analyzed by SDS-PAGE under reducing conditions (Figure 1), a number of polypeptides with Mr ≈ 26,000, 28,000, 31,000, 36,000, and 42,000 were precipitated by K-1-21. Similar results were obtained from another KMA$^+$ B lymphoblastoid cell line (MW-28; data not shown). Cell surface IgG, precipitated by protein A from the same HMy2 cell lysates (Figure 1), revealed reduced subunits with identical electrophoretic mobilities to those present in KMA, plus an additional subunit of Mr ≈ 64,000. When electrophoresed under nonreducing conditions, the Mr 64,000 and Mr 26,000 subunits present in the reduced protein A precipitate were replaced by a single component of Mr ≈ 160,000, indicating that they represent the heavy and light chains, respectively, of IgG.

The Mr ≈ 64,000 Ig heavy chain was not precipitated by antibody K-1-21. The independence of KMA from heavy chain was confirmed by first incubating ^{125}I-labeled HMy2 lysates with protein A-Sepharose before immunoprecipitation with K-1-21. As a control for nonspecific preclearing effects, lysates were also preincubated with HGG-Sepharose. Figure 2 shows that the KMA-associated polypeptides were not removed by prior precipitation of IgG from the cell lysates.

Identity of the Mr ≈ 26,000 subunit. The Mr ≈ 26,000 subunit of KMA exhibited an identical apparent m.w. to the light chain component of membrane IgG from HMy2 cells. To confirm the identity of these two polypeptides, the Mr ≈ 26,000 bands from each track were excised and were subjected to limited digest peptide mapping with staphylococcal V8-protease. The patterns of digestion

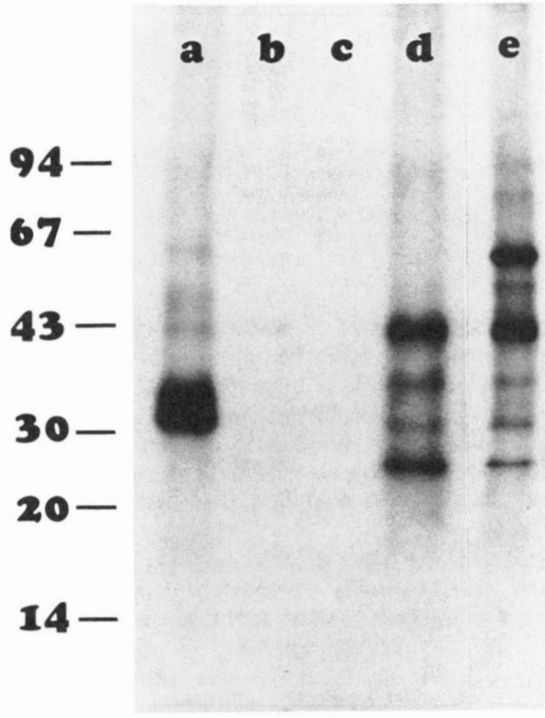

Figure 1. Autoradiograph of SDS gradient gel electrophoresis of reduced immunoprecipitates obtained from ^{125}I-HMy2 cell lysates precipitated with: (a) WM-2; (b) F-1-1 (-ve control); (c) K-1-21 + κ-BJP (-ve control); (d) K-1-21 + λ-BJP; (e) protein A.

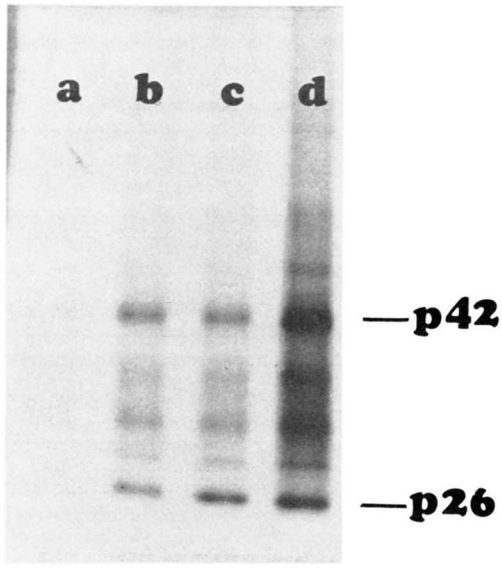

Figure 2. Autoradiograph of SDS gradient gel electrophoresis of reduced immunoprecipitates prepared either directly from ^{125}I-HMy2 cell lysates (a, d) or cell lysates preabsorbed with protein A (c) or HGG-Sepharose (b). Samples b, c, and d were precipitated with K-1-21 + λ-BJP; sample "a" (-ve control) was precipitated with K-1-21 + κ-BJP.

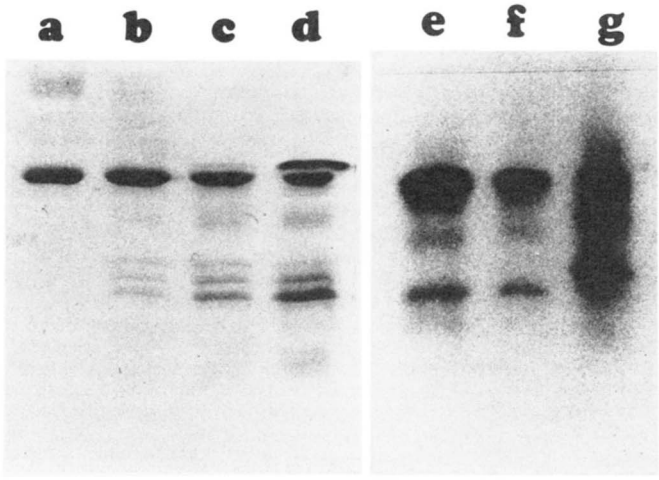

Figure 3. Limited digest peptide maps of p26 (e, f) and p28 (g) from ^{125}I-labeled immunoprecipitates and a κ-BJP (a to d). Digestion was carried out as described in *Materials and Methods*, at: 0 (a); 1 (b); 10 (c, e, f, g); and 100 (d) μg/ml. p26 (e), and p28 (g) were obtained by immunoprecipitation with K-1-21; p26 (f) was obtained by immunoprecipitation with protein A. Tracks a to d were stained with Coomassie Blue; tracks e, f, and g, were autoradiographed.

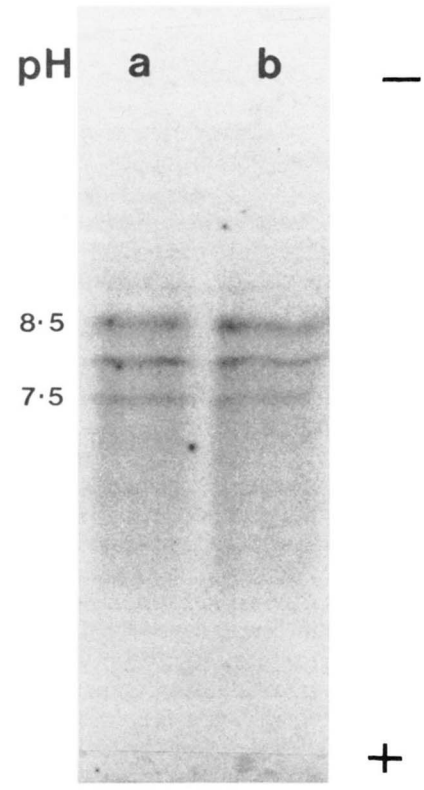

Figure 4. Autoradiograph of IEF patterns obtained for p26 obtained from (a) K-1-21 immunoprecipitate and (b) protein A immunoprecipitate.

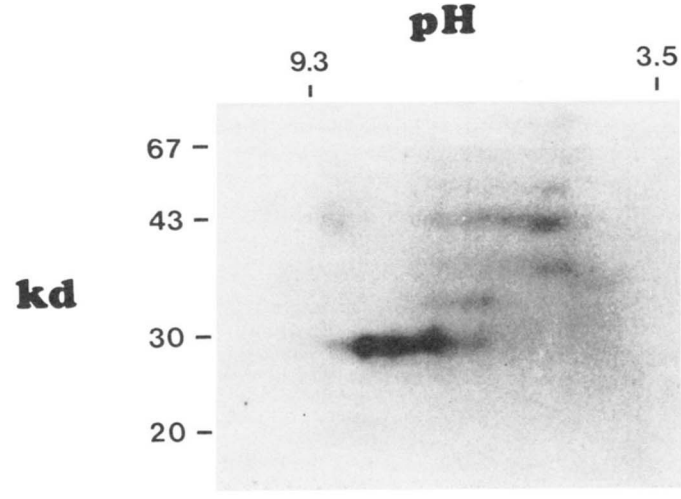

Figure 5. Two-dimensional electrophoretic separation of the K-1-21-specific immunoprecipitate from ^{125}I-HMy2 cell lysate. Separation in the first dimension was by IEF, followed by SDS-gradient gel electrophoresis in the second dimension. Autoradiography was performed as described in *Materials and Methods*.

were identical (Figure 3) and exhibited a high degree of homology with the digestion pattern of a purified κ-BJP.

The isoelectric points of the two Mr 26,000 components were also compared. Strips containing the polypeptides were excised from a dried 10% acrylamide SDS gel, were rehydrated, and were electrofocused on a horizontal slab IEF gel. As shown in Figure 4, the isoelectric points were identical.

Identity of the Mr ≈ 42,000 subunit. The Mr ≈ 42,000 protein noncovalently associated with the KMA light chains was suspected to be actin, because it co-migrated with a 42,000 dalton species coprecipitating with cell surface IgG (Figure 1). Actin has been repeatedly demonstrated to noncovalently associate and coprecipitate with cell surface Ig (12–14). To determine the isoelectric points of the components coprecipitating with KMA, K-1-21 precipitates from HMy2 cells were analyzed by two-dimensional gel electrophoresis, with IEF in the first dimension followed by SDS-PAGE. As shown in Figure 5, the Mr ≈ 42,000 subunit had a marked tendency to streak in the IEF dimension, as has been observed to occur with actin after affinity purification procedures (15). The isoelectric points of the Mr ≈ 36,000, 31,000, and 28,000 subunits were pH 5.7 to 6.1, pH 6.4 to 6.6, and pH 7.5, respectively.

To further explore the possibility that the Mr ≈ 42,000 subunit was an isomer of actin, the Mr ≈ 42,000 protein coprecipitating with both KMA and cell surface IgG was excised from a dried 10% acrylamide SDS gel, and was compared with purified rabbit skeletal muscle actin by

limited digest peptide mapping with the use of staphylococcal V8-protease. The species coprecipitating with KMA and with surface IgG generated identical patterns of radiolabeled peptides (Figure 6). Moreover, these were homologous to the Coomassie Blue-stained peptides generated from skeletal muscle actin. Slight differences in the protease-digest maps of lymphocyte and skeletal muscle actins have been described (16) and would be expected, considering the difference in isomers and, to a lesser extent, species (17).

Cell surface origin of precipitated KMA. Because free light chains are present intracellularly in many normal and transformed B lymphocytes (18), and because actin is an ubiquitous intracellular protein (17), it was essential to exclude the possibility of intracellular radiolabeling. Cell viability was monitored in all experiments before and after radiolabeling by staining with acridine orange and ethidium bromide (6). Viability was always greater than 95% and usually 97%. The possibility of radioiodination catalyzed by endogenous cellular peroxidase was tested by radiolabeling two identical aliquots of HMy2 cells but omitting lactoperoxidase from one reaction mixture. As shown in Table I, radiolabeled material specifically precipitated by K-1-21 was not detectable if lactoperoxidase was omitted in the labeling procedure.

Finally, one aliquot of radioiodinated HMy2 cells was treated with pronase, while the other was mock-treated. Cell viability in both treatment groups was greater than 95%, and after washing, lysis, and immunoprecipitation (Table I), specifically precipitated radioactivity was absent from cells treated with pronase.

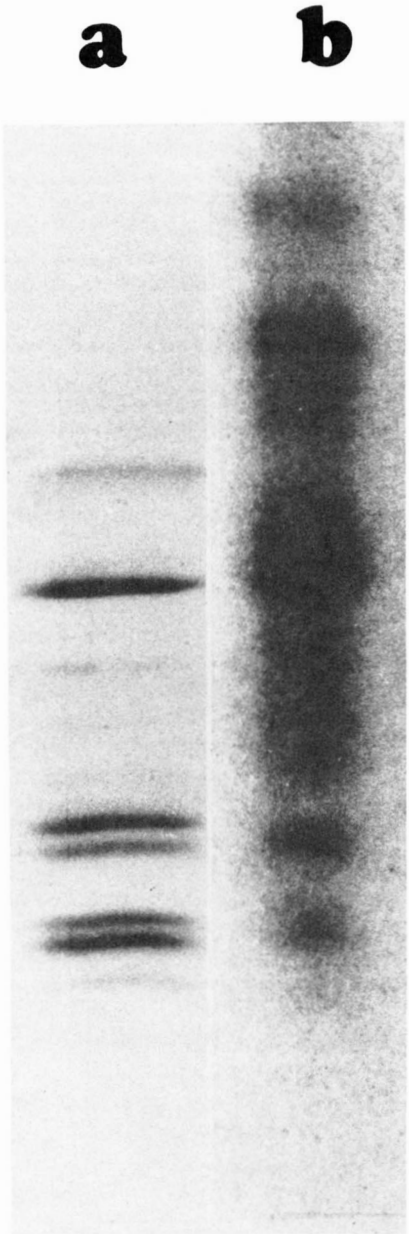

Figure 6. Limited peptide digest maps of p42 (b) and rabbit skeletal muscle actin (a). p42 was excised after SDS-gel electrophoresis of a K-1-21 immunoprecipitate. Both samples were digested in the sample well with staphylococcal V8 protease at 100 μg/ml before electrophoresis on an SDS-gradient gel. Track "a" was stained with Coomassie Blue; track "b" was autoradiographed.

DISCUSSION

The monoclonal antibody K-1-21 recognizes an antigenic determinant present on free κ-light chains, and also an antigen (KMA) on the cell surface of some B lymphoid tumors (1). The present report describes the structure of KMA in immunoprecipitates from HMy2 and MW-28 cells, two KMA^+ B cell lines. Analysis of immunoprecipitates by SDS-PAGE under reducing conditions revealed a predominant component co-migrating with Ig light chain (Figure 1). Identity of the Mr ≈ 26,000 component with Ig-associated κ-chains was established by IEF and limited peptide digest mapping of the component isolated from polyacrylamide gels (Figures 3 and 4). The failure of protein A treatment to remove KMA from radiolabeled cell lysates while effectively precipitating intact IgG (Figure 2) strengthened the conclusion that the K-1-21-defined cell surface epitope resides in non-heavy chain-associated κ-light chains. The possibility that the free κ-chain component seen on SDS-PAGE was due to intracytoplasmic labeling of the free κ-chain pool was excluded by the dependence of specific labeling on lactoperoxidase catalysis and the removal of specifically precipitable material from the cell surface by mild pronase treatment (Table I).

The existence of several other protein components in K-1-21-precipitable material suggests that KMA consists of a noncovalent complex between free κ-light chains, a cell surface form of actin, and three weakly labeled polypeptides. Of the latter, the Mr ≈ 28,000 subunit may be a minor species of free light chain exhibiting variation in the degree of glycosylation to the Mr ≈ 26,000 light chain. This is suggested by the similarity of the isoelectric points of Mr 28,000 and Mr 26,000 (Figure 5) and limited digest peptide maps (data not shown).

The mode by which free light chains could be bound to the plasma membrane is of interest. Capping studies (2) indicate that KMA is an integral membrane antigen.

TABLE I

Cell surface origin of ^{125}I-KMA

Expt.	Radiolabeling Conditions	Precipitation Protocol	% Maximal Precipitation
1	Normal	K-1-21 + λ-BJP	100
		K-1-21 + κ-BJP	28
	Lactoperoxidase omitted	K-1-21 + λ-BJP	9
		K-1-21 + κ-BJP	6
2	Normal	K-1-21 + λ-BJP	100
		K-1-21 + κ-BJP	25
	Pronase treatment Post-labeling	K-1-21 + λ-BJP	7
		K-1-21 + κ-BJP	6

However, aside from the leader peptide, κ-light chains normally lack an exposed hydrophobic sequence through which they may directly intercalate in the lipid bilayer (19). Although mutant light chains might arise as a result of aberrant κ-gene rearrangement (20) or through chromosomal translocations involving cellular oncogenes (21, 22), the light chain subunit of KMA appears to be biochemically normal (Figures 1, 3, and 4). We therefore favor a model in which the free light chains are indirectly associated with the lipid bilayer through noncovalent interaction with one or several of the other components coprecipitating with KMA, and in particular with actin.

The basis for the observed interaction between free light chains and actin is unclear. There is extensive evidence for noncovalent interactions between intact Ig and actin (12, 13, 23, 24), and indeed actin specifically coprecipitates with membrane IgG from HMy2 cells (Figure 1). Interactions between actin and other members of the Ig superfamily such as MHC Class I antigens and Thy-1 (25–27) have also been observed. It is not clear whether these interactions depend on actin-binding proteins such as Gc-protein (14), nor is it clear whether the interactions reflect a general property of the Ig domain or merely the "stickiness" of actin. The data presented here do little to resolve these questions, except that the Mr ≈ 55,000 Gc-protein is apparently not present in the KMA immunoprecipitate to contribute to actin-light chain interaction.

One interpretation of the above findings is that KMA expression on certain lymphocytes may be a consequence of the cell membrane expression of actin. Actin has been demonstrated on the surface of normal B lymphocytes in the mouse, both by lactoperoxidase-catalyzed iodination (14, 24, 28) and by immunofluorescence (29). However, Bachvaroff et al. (30) reported that actin is only detectable on the surface of normal human B lymphocytes after in vitro polyclonal activation with pokeweed mitogen. This apparent expression of cell surface actin as a function of B cell activation or differentiation could be in accordance with the observed restriction of KMA expression to B cell tumors with the phenotype of lymphoblasts, plasmablasts, or plasma cells (1) and to in vitro activated B cells. Furthermore, it may explain the observed correlation between KMA expression and stage of cell cycle in HMy2 cells (2). Thus, the appearance of KMA as a differentiation marker may reflect the expression of actin on the cell surface. This relationship is worthy of further investigation.

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