Isolation and Characterization of a 37,000-Dalton Protein Associated with the Erythrocyte Membrane*

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We have purified a 37,000-dalton polypeptide (p37) from the red cell membrane that was found in previous studies to undergo a lineage-specific alteration in its membrane association. Our data suggest that p37 associates with the red cell membrane through electrostatic interactions that are resistant to 0.5 M NaCl or 10 mM EDTA. Conditions found to elute p37 from red cell ghosts include H2O at pH 12, 0.1 M NaOH + 1 mM ethanol and 1.0% Triton X-100. p37 was purified substantially from ghosts by Triton X-100 solubilization followed by sequential DEAE-Sephadex and CM-Sephadex chromatography.

When p37 was analyzed by two-dimensional gel electrophoresis, a family of isoelectric focusing variants was detected ranging in pl from 7.0 to 7.8. All of the isoelectric focusing variants showed homology to one another when compared serologically with anti-p37 antibodies or by limited peptide mapping using Staphylococcus aureus V8 protease. The isoelectric focusing variants appear to represent distinct, yet related polypeptides rather than degrees of post-translational modifications to a single species, as much as all of the variants are present in anti-p37 immunoprecipitates prepared from in vitro translation programmes with p37 mRNA.

Hematopoiesis is characterized by complex patterns of gene expression ultimately resulting in numerous types of highly differentiated blood cells. One of the hallmarks of erythroid differentiation is the activation of the hemoglobin gene complex with a corresponding synthesis and accumulation of globin protein. In addition to hemoglobin, the lineage displays specific changes in the composition and organization of the cell membrane that are unique to erythroid cells (1, 2). Red cells express several membrane markers that are lineage-specific, including glycophorin, band III, and spectrin. The red cell membrane also lacks many proteins common to other cells of the body such as histocompatibility antigens (3). Many of the changes occurring at the membrane level in maturing erythroid cells have been reviewed by Sieff et al. (3).

We recently identified another developmental change occurring at the membrane level during erythroid differentiation (4, 5). A membrane-associated protein of Mr 37,000 (p37) was detected and its post-translational processing investigated (4, 5). The protein is ubiquitous among different human hematopoietic cell types, but appears to interact with the plasma membrane of erythroid cells in a lineage-specific manner (4). In cells with an erythroblastic phenotype, as well as in cells of other lineages, p37 appears to be expressed outside of the cell. In reticulocytes and mature erythrocytes, however, p37 is confined to the inner membrane surface. In the work presented here, p37 has been purified from the red cell membrane and characterized by two-dimensional gel electrophoresis. The results show that p37 isolated from K562 erythroleukemic cells and erythrocytes are identical molecules (see Miniprint section). Analysis of p37 molecules from K562 cells and from ghosts by two-dimensional gel electrophoresis reveals the existence of a family of p37 polypeptides, related immunologically and biochemically, yet differing from one another in isoelectric point. The results suggest the possibility that the isoelectric focusing variants of p37 represent distinct polypeptides translated from separate messenger RNAs.

MATERIALS AND METHODS

Cell Culture and Biosynthetic Labeling—K562 cells were maintained at 37 °C in a humidified atmosphere of 5% CO2, 95% air in RPMI 1640 medium (K. C. Biological, Lenexa, KS) supplemented with 10% heat-inactivated horse serum (HyClone Laboratories, Logan, UT). K562 cells display characteristics of an erythroid progenitor cell. In reticulocytes and mature erythrocytes, however, p37 is confined to the inner membrane surface. In the work presented here, p37 has been purified from the red cell membrane and characterized by two-dimensional gel electrophoresis. The results show that p37 isolated from K562 erythroleukemic cells and erythrocytes are identical molecules (see Miniprint section). Analysis of p37 molecules from K562 cells and from ghosts by two-dimensional gel electrophoresis reveals the existence of a family of p37 polypeptides, related immunologically and biochemically, yet differing from one another in isoelectric point. The results suggest the possibility that the isoelectric focusing variants of p37 represent distinct polypeptides translated from separate messenger RNAs.

Detergent Lysis and Immunoprecipitation—A detailed description of our immunoprecipitation protocol has been published previously (4, 5). In brief, when labeled cells were immunoprecipitated, they were first solubilized in Triton X-100 and the lysate was clarified by ultracentrifugation. In vitro translation products were also immunoprecipitated in the presence of Triton X-100. Immune complexes were removed from solution with protein A bearing Staphylococcus aureus bacteria (8), which were then washed repeatedly in buffer containing 0.2% SDS. Immune complexes were eluted into SDS sample buffer (10) during a 5-min incubation in boiling water. The antisera used were raised in rabbits against: (a) red cell ghosts prepared according to Dodge et al. (9), and (b) p37 purified from ghosts as described in the text. Polyacrylamide Gel Electrophoresis—One-dimensional SDS gels were of 10 or 12.5% acrylamide and were prepared and run according to Laemmli (10).

Two-dimensional gel electrophoresis was performed according to O'Farrell (11) employing pH 3.5–10 Ampholines (LKB or Sigma) in the focusing step. The second dimension SDS gels were of 10 or 12.5% acrylamide and were of the Laemmli type.

After electrophoresis, gels were stained with Coomassie Blue. In

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pared from labeled K562 cells with anti-red blood cell ghost antiserum. Immunoprecipitates were electrophoresed in the first dimension on isoelectric focusing gels containing pH 3.5-10 Ampholines. Following focusing, the tube gels were incubated in SDS sample buffer containing 2-mercaptoethanol and pressed on top of 12.5% SDS-PAGE gels. Following electrophoresis in the second dimension, gels were stained, destained, and processed for autoradiography. Molecular weights in the left margin are $10^3$.

some cases, destained gels were impregnated with ENHANCE (New England Nuclear). For autoradiography, gels were dried and exposed to pre-flashed x-ray film (Kodak XAR) for 5–10 days according to the procedures of Bonner and Laskey (12).

One-dimensional Peptide Mapping—One-dimensional peptide mapping was performed as described by Cleveland et al. (13). The p37 molecules to be mapped (either unlabeled or labeled with $^{35}S$ methionine) were excised using a scalpel from SDS-PAGE gels that had been briefly stained with Coomassie Blue and then destained with $H_2O$ at 4 °C to visualize the bands.

p37 samples excised from SDS gels were digested with 2 µg of S. aureus V8 protease (Boehringer Mannheim) as described by Cleveland et al. (13). Peptides generated by V8 digestion were electrophoresed in 15% polyacrylamide gels which were stained with either Coomassie Blue or silver. In cases where labeled p37 molecules were mapped with V8, gels were impregnated with ENHANCE and exposed to x-ray film as described above.

RESULTS

Analysis of p37 by Two-dimensional Gel Electrophoresis—The biochemical and immunological properties of p37 molecules isolated from K562 cells and red cell ghosts were identical (see Miniprint section). In order to further characterize these proteins, their migration during electrophoresis on two-dimensional gels as described by O’Farrell (11) was compared. p37 isolated from either cell type migrated as a family of discreet spots spanning a pH range of approximately 7.0–7.8 (Figs. 1 and 2A). The p37 species immunoprecipitated from K562 cells tend to cluster into two general regions of the isoelectric focusing gel (Fig. 1). The more basic group is composed of a single, or limited number of species, and focuses in the pH 7.8 range. The more acidic group is composed of 5–6 spots and focuses in the pH 7.4 range. The migration of p37 purified from ghosts is similar to that from K562 cells (Fig. 2).

Portions of this paper (including part of “Results,” and Figs. 18–4S) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 85M-2930, cite the authors, and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

To insure that all of the 37-kDa spots seen in the gel were homologous, peptide mapping using S. aureus V8 protease was performed on the isoelectric focusing variants labeled 1–5 in Fig. 2A. The focusing variants yield comparable peptide maps (Fig. 2B), indicating sequence homology. Each of these maps is similar to maps generated from p37 isolated from K562 cells (Ref. 5 and Miniprint Section) and strongly suggests that the isoelectric focusing variants of p37 immunoprecipitated from K562 cells also share amino acid sequence homology. This conclusion is further supported by the finding that all of the isoelectric focusing variants of p37 are immunoprecipitated in an all-or-none fashion by five different antisera containing anti-p37 antibodies (not shown).

The molecular basis for the heterogeneity in migration of p37 during isoelectric focusing could result from the post-translational modification of p37 in a way that alters net charge. Alternatively, the isoelectric focusing variants of p37 could represent distinct, yet related, polypeptides, translated from different messenger RNAs. In the latter explanation, each p37 molecule would have a similar sequence, yet would
have sufficient differences in amino acid composition to alter isolectric point. As one means of distinguishing between these possibilities, p37 was translated in vitro from mRNA extracted from K562 cells. The p37 translation product(s) was immunoprecipitated from the mixture and analyzed by two-dimensional gel electrophoresis. p37 immunoprecipitated from the in vitro translation migrates as a family of spots (Fig. 3) exhibiting a pattern similar to that observed previously. The p37 shown in Fig. 3 was translated in the rabbit reticulocyte system. However, identical results were obtained when p37 was translated in the wheat germ system (not shown). Given that in vitro translation systems are not considered capable of modifying polypeptides post-translationally, the results in Fig. 3 suggest that the isolectric focusing variants of p37 represent related polypeptides translated from distinct mRNAs. This conclusion is also supported by our previous data showing that p37 is not post-translationally glycosylated (5).

DISCUSSION

The work presented here extends our knowledge of the molecular properties of a ubiquitous 37-kDa protein associated with the membranes of numerous hematopoietic cell types. We reported in previous studies that p37 is translated from an mRNA that constitutes 0.1–0.5% of the total A+ mRNA in K562 cells (4) and is approximately 1600 nucleotides in length (20). More recently (5), we determined that the protein is not glycosylated and contains no leader sequence that is cleaved as the molecule associates with the cell membrane. In cells of the erythroid lineage, p37 undergoes a lineage-specific change in its association with the membrane (4, 5). In erythroleukemic K562 cells, which are of the more primitive “erythroid blast” phenotype, and in all other hematopoietic cell types, p37 can be detected using immunoblotting on the outside of the cell. In mature red cells, however, p37 is confined to the inner membrane face (4, 5). The interaction of p37 with the erythrocyte membrane appears to be electrostatic in nature, yet resistant to moderate to high concentrations of NaCl (see Miniprint Section). p37 therefore differs from glyceraldehyde-3-P04 dehydrogenase in its association with the red cell membrane (15) even though the two proteins are of similar, if not identical molecular weight. In comparing the two proteins, we have found a serological distinction between p37 and glyceraldehyde-3-P04 dehydrogenase in that several different anti-p37 antisera are incapable of immunoprecipitating glyceraldehyde-3-P04 dehydrogenase activity.

We have partially purified p37 from red cell ghosts (see Miniprint section) using techniques previously used by other investigators for the purification of 34–39-kDa proteins with properties similar to p37 (16, 17, 19). The protein can be purified to a fairly high degree simply by sequential anion and cation ion-exchange chromatography. p37 purified with these techniques is calculated to constitute about 1% of the total membrane protein in red cell ghosts.

The p37 molecules present in K562 cells and in red cell ghosts appear identical in all respects. Both proteins are immunologically related, and, in addition, yield identical peptide maps with S. aureus V8 protease. We have partially analyzed p37s from a variety of cell types and find little immunological polymorphism in the molecule (not shown).

p37 displays considerable heterogeneity in its migration on two-dimensional gels. The polypeptide, immunoprecipitated either from K562 cells or purified from ghosts, migrates as a family of spots spanning a pH range from about 7.0 to 7.8. Each of the different isolectric focusing variants are related to one another as determined by V8 protease peptide mapping.

The isolectric focusing variants of p37 could represent variable degrees of post-translational processing of a single polypeptide chain. However, we know from previous work (5) that p37 is not glycosylated and therefore a varying degree of glycosylation is not the underlying cause of the isolectric focusing variants. We also believe that p37 is not phosphorylated in either K562 cells or red cells, since repeated attempts to label p37 during incubations of cells in the presence of inorganic 32P04 have failed. We have not excluded the possibility of other post-translational modifications to p37. In virtually every form considered (i.e. methylation, fatty acid acylation, acetylation, etc.), effects on PI can be envisioned.

If the two-dimensional migration of p37 is not due primarily to post-translational processing, the heterogeneity in isolectric focusing could result if each variant represented a different gene product. If the variants represent distinct polypeptides, there must be regions of highly conserved sequence, since the V8 peptide maps shown here did not reveal any heterogeneity in sequence in any of the variants. However, we used only a single protease in our mapping experiments and therefore it is quite possible that sequence heterogeneity among the focusing variants may have been missed. A variety of proteases will be needed to evaluate completely the homology of the different p37 species. The fact that p37 translated in vitro also migrates in two-dimensional gels as a family of spots firmly supports the notion that each variant represents a separate gene product, inasmuch as in vitro translation systems are generally not considered to be capable of post-translational processing.

The molecular properties of p37 are similar to other membrane-associated proteins of about M, 37,000 studied in a variety of other cell types. Numerous groups have characterized a 34–39-kDa protein in chicken cells undergoing transformation that is a phosphorylation target for the sarcoma virus-associated protein kinase pp60csrc (16–18). The chicken 37-kDa protein is similar to red cell p37 in that it is not

Purification of Human Red Cell p37

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REFERENCES


SUPPLEMENTARY MATERIAL TO "ISOLATION AND CHARACTERIZATION OF A 37K PROTEIN ASSOCIATED WITH THE ERYTHROCYTE MEMBRANE"

BY T. LIN AND R.W. ALLEN

RESULTS

EXTRACTION AND PURIFICATION OF P37 FROM RED CELL GHOSTS

Prior to initiating the purification of p37 from ghosts, experiments were performed to characterize the nature of the interaction of the protein with the red cell membrane. Ghosts were subjected to elution regimens previously shown by others (1,2,14) to result in the selective release of subpopulations of membrane proteins. Ghosts, following a given treatment, were incubated with anti-p37 antibodies and then the absorbed antiserum samples were used to immunoprecipitate radiolabeled p37 present in detergent lysates of labeled K562 cells. Immunoprecipitates were analyzed by SDS-PAGE and autoradiography. The presence (or absence) of p37 in a given immunoprecipitate indicated that p37 was eluted (or retained, respectively) from the red cell membrane. The results of one such experiment are shown in Figure 1 and reveal that concentrations of NaCl as high as 0.5M did not elute p37 from the red cell membrane. This concentration of NaCl quantitatively elutes many peripheral membrane proteins from ghosts (14,15). Thus, p37 appears to be rather tightly associated with the red cell membrane. The only conditions that eluted significant p37 from the membrane were alkaline pH and non-ionic detergent. The elution of p37 from the membrane under conditions of alkaline pH suggests that the interaction of the protein with the membrane is mediated primarily through electrostatic interactions that are resistant to NaCl. It should also be noted that p37 was not eluted from ghosts treated with EDTA (not shown) indicating that divalent cations are not involved in its association with the red cell membrane.


FIGURE 1: IMMUNOABSORPTION OF ANTI-P37 ANTIBODIES BY GHOSTS TREATED WITH VARIOUS ELUTING AGENTS.
25% OF PACKED GHOSTS (PREPARED ACCORDING TO DODGE ET AL. AND WE) INCUBATED FOR 30 MINUTES ON ICE. THE GHOSTS WERE PELLETED BY CENTRIFUGATION AT 12,000 × G FOR 20 MINUTES IN A MICROCENTRIFUGE. THE PELLET WAS WASHED 2X WITH 5MM NaH₂PO₄ BUFFER PH 8.0 (I.E., HEMOLYSIS BUFFER, SP8) AND THEN RESUSPENDED IN 50UL OF SP8 CONTAINING 5% OF ANTI P-37 ANTISEM. THE MIXTURE WAS INCUBATED ON ICE FOR 2 HOURS AND THEN THE GHOSTS WERE PELLETED BY CENTRIFUGATION. THE SUPERNATANT WAS ALIQUOT OF NON-IONIC DETEGENT LYSATE OF 35S-METHIONINE GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (G3PO) THROUGH LABELED K562 CELLS CONTAINING APPROXIMATELY 3×10⁶ CPM OF TCA OF CELL TYPES WITH PROPERTIES SIMILAR TO P37 (16-19). PRIOR TO TECHNIOUES PREVIOUSLY USED TO PURIFY 37K PROTEINS FROM PRECIPITABLE RADIOACTIVITY. THE LINES REFLECT POLYPEPTIDES IMMUNOPRECIPITATED BY: LANE A - NORMAL HABIT SERUM, LANE B - UNABSORBED ANTISEM, LANE C - ANTISEM ABSORBED WITH GHOSTS TREATED WITH 1% TRITON X-100, LANE D - ANTISEM ABSORBED WITH GHOSTS TREATED WITH 0.1N NaOH - 1% ETHANOL, LANE E - ANTISEM ABSORBED WITH GHOSTS TREATED WITH 0.2M NaCl AT PH 12, LANE F - ANTISEM ABSORBED WITH GHOSTS TREATED WITH SP8 CONTAINING 0.5M NaCl. SIMILAR RESULTS TO LANE F WERE OBTAINED WITH GHOSTS INCUBATED IN 10M EDTA. THE PRESENCE OF P37 IN ANY LANE INDICATES THAT THE PROTEIN WAS STRIPPED FROM THE GHOSTS BY A GIVEN TREATMENT AND THEREFORE WAS NOT ABSORB ANTI-P37 ANTIBODIES FROM THE ANTISEM. MOLECULAR WEIGHT STANDARDS ARE SHOWN IN THE LEFT MARGIN AND ARE X 10⁷.

OUR STRATEGY FOR PURIFYING P37 FROM GHOSTS WAS BASED UPON TECHNIQUES PREVIOUSLY USED TO PURIFY 37K PROTEINS FROM A VARIETY OF CELLS PROTEINS SIMILAR TO P37 (16-19). PRIOR TO SOLUBILIZATION OF THE MEMBRANE, GHOSTS WERE STRIPPED OF THE RED CELL MEMBRANE AND CAN BE QUANTITATIVELY ELUTED FROM THE MEMBRANE WITH 0.2-0.5M NaCl (15). THE STRIPPED MEMBRANES WERE USED AS A SOURCE OF P37 AND WERE SOLUBILIZED IN 10 M NaH₂PO₄ PH 7.8 CONTAINING 1% TRITON X-100 AND 2M PMSF. THE CYTOSKELETON WAS PELLETED BY CENTRIFUGATION AT 100,000×G AND THE SUPERNATANT WAS PASSED OVER A DEAE-SEPHADEX COLUMN EQUILIBRATED IN 10M NaH₂PO₄ PH 7.8 AND 10% GYCOL. APPROXIMATELY 50% OF THE PROTEIN APPLIED TO THE COLUMN FLOWED THROUGH. THE PH OF THE NON-BOUND FRACTION WAS LOWERED TO PH 6.0 WITH PHOSPHORIC ACID AND THEN WAS APPLIED TO A COLUMN OF CM-SEPHADEX EQUILIBRATED IN 10M NaH₂PO₄ PH 6.0 CONTAINING 1% GYCOL. ELUTION OF THE COLUMN WITH A 140ML LINEAR 0-0.5 M GRADIENT OF NaCl IN 10 M NaH₂PO₄ PH 6.0 YIELDED TWO MAIN SEVERAL MINOR PEAKS OF U.V. ABSORBING MATERIAL (FIGURE 2). P37 WAS ELUTED FROM THE COLUMN WITH ABOUT 0.2 M NaCl AT PH 6.0 (FIG. 2, INSET). ENRICHED FRACTIONS OF P37 WERE ABOUT 70-90% HOMOGENEOUS WHEN JUDGED BY SDS-PAGE AND STAINING WITH COOMASSIE BLUE OR SILVER (FIGURE 2, INSET). P37 PURIFIED IN THIS MANNER CONSTITUTES ABOUT 15 OF THE TOTAL RED CELL MEMBRANE PROTEIN.

FIGURE 2: PURIFICATION OF P37 FROM THE RED CELL MEMBRANE. RED CELL GHOSTS WERE PREPARED BY HYPTONIC LYSIS (9). GHOSTS WERE INCUBATED IN 20 VOLUMES OF HEMOLYSIS BUFFER (SP8) CONTAINING 0.2M NaCl FOR 20 MINUTES ON ICE TO STRIP GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (G3PD) FROM THE MEMBRANE WHICH IS OF SIMILAR SIZE TO P37. STRIPPED GHOSTS WERE WASHED 3X WITH SP8 CONTAINING 0.2M NaCl AND 2X WITH SP8 ALONE. WASHED MEMBRANES WERE THEN SOLUBILIZED IN 20ML OF 10M NaH₂PO₄ PH 7.8 CONTAINING 1% TRITON X-100 AND 2M PMSF. THE LYSATE WAS CENTRIFUGED AT 100,000 X G FOR 60 MINUTES AND THE SUPERNATANT CONTAINING ABOUT 50MG OF PROTEIN WAS PASSED SEQUENTIALLY OVER 10 ML COLUMNS OF DEAE-AND CM-SEPHADEX AS DESCRIBED IN THE TEXT. 2ML FRACTIONS WERE COLLECTED, THE A₂₈₀ MEASURED, AND SELECTED FRACTIONS WERE ANALYZED BY SDS-PAGE. MOLECULAR WEIGHT MARKERS FOR THE SDS-GEL ARE THE SAME AS IN FIGURE 1.

SEVERAL EXPERIMENTAL APPROACHES WERE TAKEN TO INSURE THAT THE P37 ISOLATED FROM GHOSTS IS THE SAME PROTEIN WE PREVIOUSLY CHARACTERIZED IN K562 ERYTHROLEUKEMIC CELLS (5). ONE APPROACH WAS TO DETERMINE WHETHER OR NOT P37 FROM THE DIFFERENT SOURCES WERE IMMUNOLOGICALLY RELATED. A COMPETITION ASSAY WAS PERFORMED IN WHICH A SUPPLEMENT OF P37 ISOLATED FROM GHOSTS, WAS MIXED WITH AN ALIQUOT OF ANTI-RBC ANTISEM AND ALLOWED TO INCUBATE OVERNIGHT AT 4°C. THE MIXTURE WAS SUBSEQUENTLY ADDED TO P37 PRESENT IN A DETERGENT LYSATE FROM (55S) METHIONINE LABELED K562 CELLS. IMMUNE COMPLEXES WERE ISOLATED AND ANALYZED BY SDS-PAGE AND AUTORADIOGRAPHY. AS SHOWN IN FIGURE 3, P37 PURIFIED FROM GHOSTS EFFECTIVELY COMPETED WITH P37 FROM K562 CELLS FOR ANTIBODY BINDING INDICATING THAT THE PROTEINS CROSS-REACT IMMUNOLOGICALLY. THE компететassi ASSAY EXHIBITS SPECIFICITY IN THAT P37 IS THE ONLY POLYPEPTIDE LACING IN THE IMMUNOPRECIPITATE PREPARED WITH ANTISEM TO WHICH UNLABELED P37 HAD BEEN ADDED.
ABILITY OF P37 PURIFIED FROM GHOSTS TO IMMUNOLOGICALLY COMPETE WITH P37 FROM K562 CELLS FOR ANTIBODY BINDING. P37 WAS EXTRACTED FROM GHOSTS AND PARyially purified as described. 18ug of p37 and 3ul of anti-RBC antiserum were added to 100ug of PBS and allowed to incubate overnight at 4°C with gentle mixing. The mixture was then added to an aliquot of \(^{[35S]}\)-METHIONINE LABELED DETERGENT LYSATE FROM K562 CELLS. IMMUNOPRECIPITATES WERE PREPARED AND ANALYZED BY SDS-PAGE AND FLUOROGRAPHY. PRODUCTS IMMUNOPRECIPITATED WITH: LANE A - NORMAL RABBIT SERUM, LANE B - ANTI-RBC ANTISERUM INCUBATED OVERNIGHT WITH PBS ALONE, LANE C - ANTI-RBC ANTISERUM INCUBATED WITH PBS CONTAINING 18ug OF PURIFIED P37. MOLECULAR WEIGHT MARKERS ARE AS IN FIGURE 1.

THE P37 POLYPEPTIDES FROM K562 CELLS AND GHOSTS WERE ALSO COMPARED BY LIMITED PEPTIDE MAPPING EMPLOYING THE METHODS OF CLEVELAND ET AL (13). STAPH V8 PROTEASE WAS USED TO GENERATE THE PEPTIDE MAPS SHOWN IN FIGURES 3 AND 4. IT IS CLEAR FROM THE DATA IN FIGURES 3 AND 4 THAT P37 PURIFIED FROM GHOSTS AND THAT IMMUNOPRECIPITATED FROM K562 CELLS ARE HOMOLOGOUS.

FIGURE 3: ABILITY OF P37 PURIFIED FROM GHOSTS TO IMMUNOLOGICALLY COMPETE WITH P37 FROM K562 CELLS FOR ANTIBODY BINDING. P37 WAS EXTRACTED FROM GHOSTS AND PARyially purified as described. 18ug of p37 and 3ul of anti-RBC antiserum were added to 100ug of PBS and allowed to incubate overnight at 4°C with gentle mixing. The mixture was then added to an aliquot of \(^{[35S]}\)-METHIONINE LABELED DETERGENT LYSATE FROM K562 CELLS. IMMUNOPRECIPITATES WERE PREPARED AND ANALYZED BY SDS-PAGE AND FLUOROGRAPHY. PRODUCTS IMMUNOPRECIPITATED WITH: LANE A - NORMAL RABBIT SERUM, LANE B - ANTI-RBC ANTISERUM INCUBATED OVERNIGHT WITH PBS ALONE, LANE C - ANTI-RBC ANTISERUM INCUBATED WITH PBS CONTAINING 18ug OF PURIFIED P37. MOLECULAR WEIGHT MARKERS ARE AS IN FIGURE 1.

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