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Purification of *HLA*-linked B Lymphocyte Alloantigens in Immunologically Active Form by Preparative Sodium Dodecyl Sulfate-Gel Electrophoresis and Studies on Their Subunit Association*

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The *HLA*-linked B cell alloantigen (p29,34) is composed of two subunits of 29,000 (p29) and 34,000 (p34) molecular weight. The partially purified *HLA*-linked B cell alloantigen was purified by a final step of preparative sodium dodecyl sulfate-gel electrophoresis. An antiserum was prepared against p29,34 which specifically lysed B lymphocytes. In sodium dodecyl sulfate at 21°, p29 and p34 remained noncovalently associated and retained immunologic activity; subunit dissociation at higher temperatures correlated with loss of immunologic activity. Although the pI values of p29 and p34 are 6.1 and 5.2, respectively, the subunits co-electrofocus under nondenaturing conditions. In addition, cross-linking studies showed the B cell antigen has a $(p29)_1(p34)_1$ subunit structure.

A B lymphocyte-specific alloantigen has been identified in humans which is specified by a genetic locus closely linked to *HLA-A* and *-B* (1-16) and appears to be homologous to mouse and guinea pig Ia antigens (1, 12). This alloantigen is specified by a locus either closely linked to or identical with the *HLA-D* locus, the strongest mixed lymphocyte culture stimulation locus, since (*a*) in a cross-over between *HLA-D* and *HLA-B*, the alloantigen is associated with *HLA-D* (4); (*b*) typing with certain B cell alloantisera has given good agreement with typing for *HLA-D* using homozygous stimulator cells (4-7); and (*c*) mixed lymphocyte culture stimulation can be inhibited by antisera against the B cell-specific antigen (4, 6, 13, 15, 16). Since these B lymphocyte antigens are *HLA-D*-linked, but not necessarily products of *HLA-D*, and are identified serologically rather than by the mixed lymphocyte culture reaction, they will be called in this report *HLA*-linked B lymphocyte alloantigens or, for brevity, B cell antigens or p29,34. Heteroantisera to B cell-specific antigens appear to have the same properties as the antisera raised in humans, except for a lack of allospecificity (14-16). In immunoprecipitation experiments with radiolabeled, detergent-solubilized antigens, human B cell-specific and murine Ia antisera generally precipitate two polypeptides with molecular weights between 25,000 and 35,000 (15-20). A papain-solubilized B cell-specific antigen containing polypeptide chains of M_r 23,000 and 30,000 (p23,30) has been purified (14, 15). The detergent-solubilized B cell-specific antigen (p29,34) contains polypeptide chains of M_r 29,000 (p29) and 34,000 (p34) and these subunits have been isolated under denaturing conditions by preparative sodium dodecyl sulfate-gel electrophoresis and chemically characterized (12). They have NH_2-terminal isoleucine and glycine, respectively, and have unique NH_2-terminal sequences. However, their amino acid compositions are highly similar, and peptide mapping studies showed them to have a high degree of homology to one another (12).

This report describes the purification of the detergent-soluble *HLA*-linked B cell alloantigen in immunologically intact form, the preparation of an antiserum to it, characterization for B cell specificity, studies on subunit association and its correlation with immunologic activity.

METHODS

Purification of B Cell Antigens—B cell antigens were partially purified together with *HLA*-A, B antigens as described previously (21-23). Complete purification was achieved by a final preparative SDS[1]-gel electrophoresis step. Pool B (40 ml) (see Refs. 12 and 22) from Bio-Gel A-5m filtration (0.74 mg/ml) was concentrated and the excess of Brij 99:97 (Brij 99:Brij 97, 2:1) detergent was removed by absorption of protein to 10 ml of DEAE-cellulose DE52 (Whatman) equilibrated with 10 mM Tris/HCl, pH 8.2, 0.05% Brij 99:97, which was then washed with 60 ml of the above buffer and eluted with 80 ml of the same buffer containing 0.5 M NaCl. The elution fractions containing protein, judged by A_{280}, were pooled, dialyzed *versus* 1 mM Tris/HCl, pH 7.5, and concentrated with an Amicon PM-10 membrane to 2.5 ml (yield = 18.6 mg of protein or 64%). To this material were added 2 ml of 5% SDS, 20% glycerol, and 0.001% bromphenol blue, and the sample was divided in half. One-half was placed in a boiling water bath for 10 min (for preparation of dissociated p29 and p34) and the other half allowed to stand at room temperature for 1 h (for preparation of the p29,34 complex). The samples were placed on SDS slab gels which were 0.32 × 13 cm in depth and width. The lower gel (10% polyacrylamide) was 20 cm in height and the upper gel was 3 cm in height. Electrophoresis was at 100 V until the dye reached the bottom. Guidestrips 1-cm wide were cut from the edges and center of the gel, stained with Coomassie blue (24), and after correc-

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[1] The abbreviation used is: SDS, sodium dodecyl sulfate.

tion for expansion during staining, the areas containing protein were cut from the unstained portion of the gel.

Immunization – For inoculation of rabbits one-tenth by weight of the appropriate gel slice (containing ~200 μg of protein) per injection was minced, homogenized with salt/P_i (Dulbecco's phosphate-buffered saline, 0.20 g/liter of KCl, 0.20 g/liter of KH_2PO_4, 0.047 g/liter of $MgCl_2$, 8.0 g/liter of NaCl, 1.15 g/liter of Na_2HPO_4) obtained from GIBCO, and Freund's adjuvant, and injected into rear footpads and subcutaneously into the back. Rabbits were bled 6 weeks later, and the antisera along with the pre-immune sera, were titered against RPMI 4265 cells.

SDS-Gel Electrophoresis – One-dimensional SDS-gel electrophoresis was as described by Studier (25). Two-dimensional electrophoresis, with isoelectric focusing in the first dimension and SDS-gel electrophoresis in the second dimension, was as described by O'Farrell (26), except in some cases urea was omitted from the first dimension. Proteins were stained with Coomassie blue using the procedure of Vesterberg (24).

Cytotoxicity Assay – Antisera were tested against B and T lymphocytes purified from peripheral blood (3) and against paired T and B cultured cell lines derived from three individuals (8). Before detergent-soluble antigen was tested for its ability to inhibit specific antisera in the cytotoxicity assay, it was preincubated with bovine serum albumin (hereafter, albumin) to prevent detergent-mediated lysis of target cells (21, 22). Cultured RPMI 4265 cells were used as targets.

RESULTS

Purification

The *HLA*-linked B cell alloantigen (p29,34) was obtained partially purified as a by-product of the purification of *HLA*-A, B antigens from RPMI 4265 lymphoblastoid cells (12, 22). Briefly, the steps were membrane preparation, detergent solubilization, lectin affinity chromatography, and agarose gel filtration. p29,34 was separated from the majority of the *HLA*-A,B antigens (p44,12) in the gel filtration step. The fractions enriched for p29,34 from this step were placed in a pool (designated Pool B). At this stage, p29 and p34 were the major constituents of Pool B as shown by SDS-gel electrophoresis.[2] On the basis of similarities to detergent-soluble murine Ia antigens (20) in their behavior during purification and their molecular weights, p29 and p34 were thought to be the human analogues of the native murine Ia antigens, *i.e.* the *HLA*-linked B cell alloantigens. However, completely purified material was required to prove this point as well as to provide material for immunization studies.

Therefore, use was made of the observation that in the presence of SDS at 21–38°, p29,34 remains associated in a complex which calibrates at an apparent M_r of 55,000 in SDS gels (12), while contaminating *HLA*-A,B antigens (p44,12) in Pool B are dissociated and well separated. It was hypothesized that this form of p29,34 in SDS retains a structure similar to that of the native molecule, and might be active as an antigen. Therefore, Pool B from agarose chromatography was incubated with SDS at 21° and the p29,34 complex was purified by preparative SDS-slab gel electrophoresis as described under "Methods," thus separating them from p44 and p12. p29 and p34 were also purified after dissociation at 100° in the presence of SDS. Portions of the gels were eluted into distilled water and lyophilized. Analytic SDS-gel electrophoresis (Fig. 1) showed the eluted proteins to be homogeneous.

Preparation of Antiserum

Separated p29 and p34 did not elicit antibodies detectable by precipitation in gel or ability to lyse lymphocytes in any of the three rabbits in which they were injected and boosted twice with 200-μg amounts. However, three rabbits injected once with the p29,34 complex (~200 μg each) produced antisera lytic for cultured B cells, and the antiserum with the highest titer was studied in detail (Table I). The antiserum was tested against three pairs of cell lines with B or T lymphocyte character, each pair having been derived from the same individual. Since human alloantisera to *HLA*-linked B cell antigen and rabbit anti-p23,30 (the papain-solubilized B cell antigen) are reactive with B but not T lymphocytes (14), it was expected that the rabbit heteroantisera to p29,34 would show a similar pattern of reactivity. Indeed, the serum had a much greater titer against the B cell than against the T cell line of each pair. The antiserum was also tested diluted 1:5 against peripheral B and T cells purified from a panel of 48 individuals (3). It was positive with B cells from 46 individuals and with T cells from only one individual, the latter reaction possibly being due to experimental error. This antiserum is therefore B cell-specific, although at low dilution it shows some reactivity with T cell lines, but not with peripheral T lymphocytes. Perhaps T cell lines contain a cross-reactive antigen or a lower density of the same antigen. Mouse Ia antisera show a similar pattern of strong reactivity with B lymphocytes and little or no reactivity with resting T cells, but a much greater degree of reactivity with concanavalin A-stimulated T cells (27).

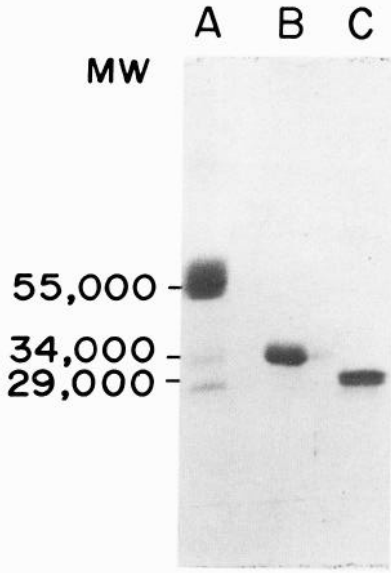

FIG. 1. Analytic SDS-gel electrophoresis of proteins eluted after preparative SDS-gel electrophoresis. After elution from preparative gels, proteins were dissolved in sample buffer containing 5% 2-mercaptoethanol and rerun on 11% polyacrylamide-SDS slab gels. *A*, p29,34 (not boiled); *B*, p34 (boiled); *C*, p29 (boiled).

SDS gel-purified p29,34 (Fig. 1*A*) not only elicited a B cell-specific antiserum but also has been shown to be the *HLA*-linked B cell alloantigen by inhibition experiments. Eluted p29,34 (as well as p23,30) completely inhibited the *HLA*-linked B cell alloantiserum Po (see Refs. 28 and 44). The specificity of this inhibition was shown by the fact that p29,34 from RPMI 4265 cells did not inhibit another *HLA*-linked B cell serum recognizing a different allospecificity and did not inhibit an *HLA*-A2 serum (compare Ref. 14).

Heat Stability of p29,34

Samples of Pool B were heated, in the presence or absence of SDS and dithiothreitol, and portions were withdrawn for SDS-

[2] See Fig. 2 for an SDS gel of Pool B and Fig. 12 of Ref. 22 for SDS gels of each gel filtration pool and an earlier purification step.

TABLE I

Lysis of B and T cultured lymphoblastoid cells with anti-p29,34 serum

Cell line	B or T cell character	Titer[a]
8392	B	250
8402	T	10
PA3	B	100
CEM	T	10
SB	B	500
HSB	T	50
4265	B	500

[a] Dilution giving 50% lysis.

gel electrophoresis (Fig. 2) and for assay of their antigenic activity (Fig. 3). After treatment at 21° and 57° in the absence of SDS, p29 and p34 remained associated in a complex which calibrated at M_r = 55,000 on SDS gels (Fig. 2) and retained antigenic activity (Fig. 3) as measured in the inhibition of cytotoxicity assay with rabbit anti-p29,34 serum. However, after treatment at 80° and 100°, most proteins aggregated and did not enter the gel despite the fact that 2% SDS was added to the samples before electrophoresis, and almost all antigenic activity was destroyed. When treatment was conducted in the presence of SDS, the M_r = 55,000 complex was present at 20°, in lesser amount at 57°, and absent at 80° and 100°, correlating with 50% retention of activity at 21°, 12% at 57°, and 0% at 80° and 100°. The presence of dithiothreitol had little effect on stability (Fig. 3). Samples were 78% stable in SDS and dithiothreitol at 0°, at which temperature the SDS precipitated, and were 82% stable to two cycles of freezing and thawing (data not shown). Therefore, in the presence of SDS, p29,34 was less stable to heating, and loss of antigenic activity was correlated with subunit dissociation. Some dissociation in SDS occurred at 21° and was of course also found in samples which had been heated in the absence of SDS but were mixed with SDS before electrophoresis, and correlated with 50% loss of activity in SDS at 21°. In a previous experiment (12), little dissociation was observed by SDS-gel electrophoresis when samples were heated with a lower concentration (0.6%) of SDS and a higher concentration of nonionic detergent at 21°, 27°, and 38°. It is possible that antigenic activity would have plateaued in this temperature range if these temperatures had been included in the present experiment in 2% SDS. However, it is also possible that stability is dependent upon the concentration of SDS. Since electrophoresis was for 11 h at 21° in the presence of 0.1% SDS, the p29,34 complex is fairly stable under these conditions.

Subunit Structure of B Cell Antigen

The above data indicated that the complex migrating at an apparent M_r of 55,000 in SDS gels is immunologically active, while dissociated p29 and p34 are immunologically inactive. Apparently, the M_r = 55,000 complex contained noncovalently associated p29 and p34. However, other interpretations concerning the origin and breakdown of the M_r = 55,000 complex were possible, and the number of p29 and p34 subunits contained in the B cell antigen was uncertain. Therefore, further experiments were carried out on the subunit structure of the B cell antigen.

Isoelectric Focusing—The isoelectric focusing properties and the association of p29 with p34 were studied using a two-

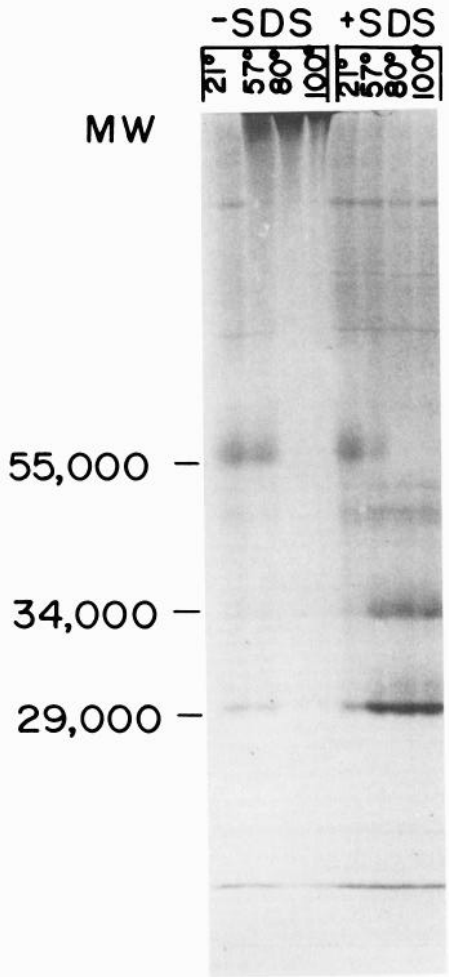

FIG. 2. Heat stability of the p29,34 complex measured by SDS-gel electrophoresis. Pool B, which contained 2% Brij 99:97, was concentrated and removed from excess detergent by ion exchange chromatography as described under "Methods." Samples were 1.2 mg of protein/ml and contained 0.2% Brij 99:97. They were heated at 20°, 57°, or 80° for ½ h or at 100° for 5 min, with no additions, or in the presence of 2% SDS or 2% SDS and 2 mM dithiothreitol (not shown), aliquots were withdrawn for assay with anti-p29,34 serum, and to the remainder, SDS was added to samples not already containing it, and to samples not containing dithiothreitol, iodoacetamide was added to a final concentration of 50 mM. SDS-gel electrophoresis was in 9 to 13% polyacrylamide slab gels.

dimensional isoelectric focusing system (26) (Fig. 4). In this system, isoelectric focusing of the lectin column eluate was conducted in a tube gel in dimension one. This tube gel was equilibrated in SDS sample buffer, and in some cases was heated at 80° for 5 min. It was then placed on a slab gel, and SDS-gel electrophoresis was conducted in dimension two. In Fig. 4, *A* and *B*, focusing was conducted in the absence of urea. p44, the large subunit of *HLA*-A,B antigens, focused as many spots smeared together, correlating with earlier findings that each *HLA*-A,B specificity exhibited several peaks of activity, possibly due to sialic acid heterogeneity (22, 29).

p29 and p34 focused in a region of the gel somewhat narrower than that in which p44 focused but in a broader region than some of the other proteins. When the equilibrated focusing gel was not heated before SDS-gel electrophoresis in the second dimension, p29,34 migrated in a complex calibrating at M_r = 55,000 (Fig. 4*A*), but when heated, migrated as dissociated p29 and p34 (Fig. 4*B*). Comparison of the positions of the

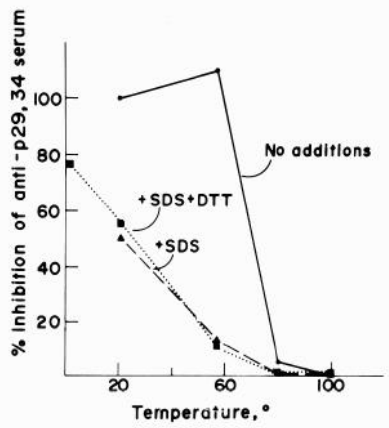

FIG. 3. Heat stability of p29,34 measured by inhibition of anti-p29,34 serum. Samples were heated in the presence of no additions, 2% SDS, or 2% SDS and 2 mM dithiothreitol (*DTT*) as described in the legend to Fig. 2 and aliquots were withdrawn for the inhibition of cytotoxicity assay using anti-p29,34 serum.

M_r = 55,000 complex in Fig. 4*A* and p29 and p34 in Fig. 4*B* lends further support to the conclusion that the M_r = 55,000 complex contains p29 and p34. Although p29 and p34 were somewhat smeared, perhaps partly due to aggregation, their positions in the focusing dimension were exactly aligned and the ratio of stain in p29 and p34 was constant in all areas. Thus, p29 and p34 remain associated during isoelectrofocusing under nondenaturing conditions.

When samples were focused in 8 M urea in the first dimension to maintain denaturation (Fig. 4*C*), p29 focused at pI = 6.1, while p34 focused at pI = 5.2. Since p29 and p34 have such different pI values under denaturing conditions, it is unlikely they would have pI values identical with one another under nondenaturing conditions, and thus it is unlikely that their association in the same region of the focusing gel under nondenaturing conditions is fortuitous.

Cross-linking Experiments—To determine the number of p29 and p34 subunits which were noncovalently associated, the B cell antigen was cross-linked with reagents containing cleavable S—S bonds, and was electrophoresed on two-dimensional SDS gels (30), unreduced in the first dimension and reduced in the second dimension (Fig. 5). Pools A and B were cross-linked with dimethyldithiobis(propionimidate) (Fig. 5, *A* and *B*) and Pool B was also cross-linked with a different cleavable reagent, dithiobis(succinimidyl-propionate) (Lomant's reagent) (31) (Fig. 5*C*). In Figs. 5, (*A*, *B*, and *C*) spots of p29 and p34 derived from a cross-linked product are seen vertically aligned with each other below the diagonal of uncross-linked proteins, showing both subunits are present in the cross-linked product. Un-cross-linked p29 and p34, on the diagonal, are also present. Cross-linking of aldolase revealed spots of tetramer, trimer, and dimer, and a similarly large amount of monomer (not shown). A somewhat higher yield of the cross-linked product was obtained with Lomant's reagent (Fig. 5*C*), although in this gel cross-linked p29 and p34 were not well resolved from each other in the second dimension. A stained tube gel, identical with the one used in the first dimension, is shown at the top of Fig. 5*C*. The M_r of the cross-linked product, as determined by calibration with standard proteins in parallel tube gels, is 64,000. This M_r is close to the sum of the subunit M_r values. Thus, p29 and p34 are associated in a $(p29)_1(p34)_1$ structure. The M_r of 64,000 is higher than the M_r of 55,000 observed for p29,34 in SDS at 21°. The p29,34 complex with an apparent M_r of 55,000 retains antigenic activity, and therefore should possess a folded conformation with electrophoretic properties which would be expected to differ from those of cross-linked, heated, unfolded p29,34.

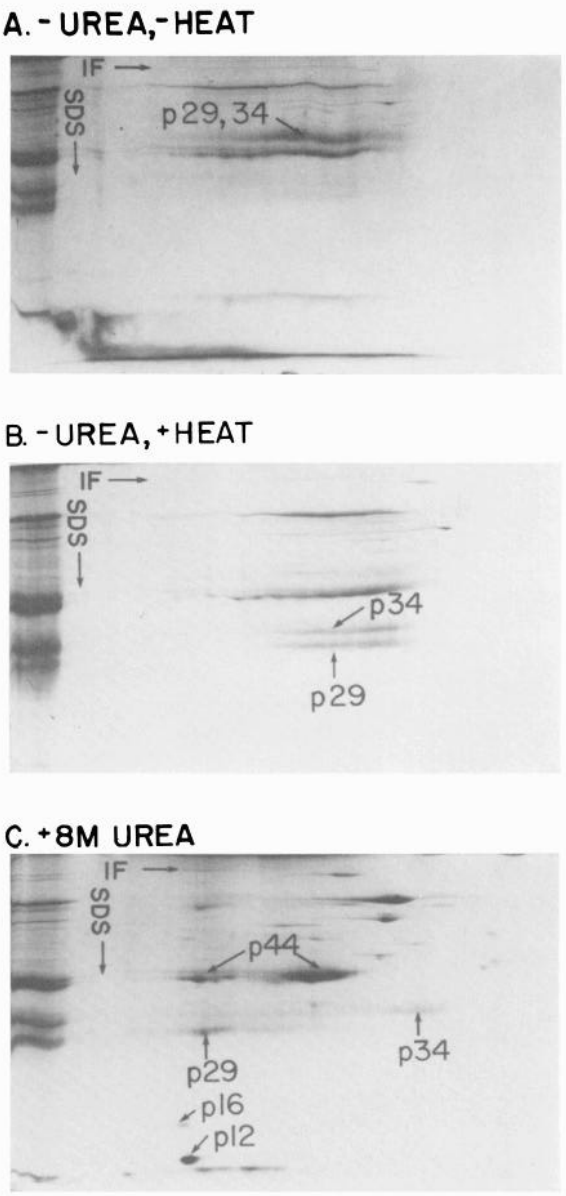

FIG. 4. Two-dimensional gels, using isoelectric focusing (*IF*) in dimension one and SDS-gel electrophoresis in dimension two, demonstrating association of p29 and p34. The lectin column eluate was separated by isoelectric focusing in dimension one, gels were equilibrated with SDS sample buffer for ½ h, and SDS-gel electrophoresis was conducted in a 10% polyacrylamide gel in dimension two, according to O'Farrell (26), with the following modifications: *A*, focusing without urea, plus 10% sucrose; *B*, same as in *A*, except after equilibration in SDS sample buffer, the tube gel was heated at 80° for 5 min; *C*, focusing in 8 M urea. A sample, which had been boiled, was applied to each second dimension gel in a slot to the left of the isoelectric focusing gel. The alkaline end of the isoelectric focusing gel is to the *left*.

$(p29)_2$ and $(p34)_2$ dimer molecules were not observed in two-dimensional gels. Since p29 and p34 were better separated from one another in the first, unreduced dimension than in the second, reduced dimension, and separation on SDS gels was linear with log M_r in this range, $(p29)_2$ and $(p34)_2$ molecules

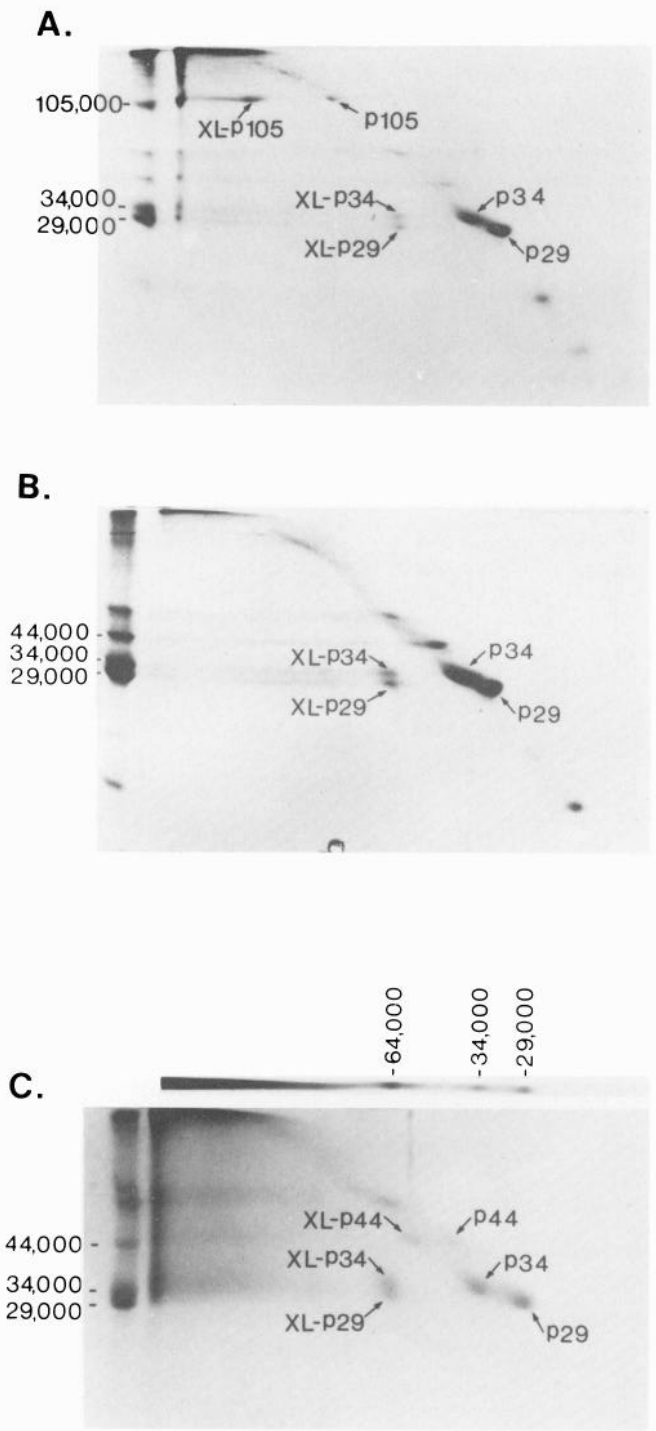

FIG. 5. Two-dimensional electrophoresis in SDS gels of cross-linked p29,34. Samples were cross-linked using modifications of the methods of Wang and Richards (30) or Lomant and Fairbanks (31) and then electrophoresed in 5% first-dimension gels, and 10% second-dimension gels essentially as described (30), except the intervening layer of agarose contained 0.1 M dithiothreitol instead of 2-mercaptoethanol, and thinner gels were used (2.5-mm tube gels, 0.8-mm slab gels). A portion of the cross-linked material was also applied to a sample slot in the second dimension to the left of the tube gel. *A*, Pool A (40 μg, 0.5 mg/ml) (22) was dialyzed *versus* 50 mM triethanolamine, pH 8.4, cross-linked with 1 mg/ml of dimethyldithiobis-(propionimidate) (30) for 1 h at 20°, the reaction was stopped with 50 mM ammonium acetate, and *N*-ethylmaleimide was added to 1 mg/ml. After 20 min acetone (5 volumes) was added, and after another hour at 0°, the precipitate was collected, dissolved in 20 μl of sample buffer, heated at 100°, and electrophoresed. *B*, Same except Pool B (60 μg, 0.74 mg/ml) was used. *C*, Pool B (140 μg, 0.7 mg/ml) was after dialysis *versus* 50 mM sodium borate, pH 9.0. Cross-linking with Lomant's reagent (31) and preparation for electrophoresis was as previously described (32). The sample was boiled in 50 μl of sample buffer, and a 20-μl portion was subjected to two-dimensional electrophoresis and a 10-μl portion was subjected to one-dimensional electrophoresis only and stained. The stained one-dimensional gel is shown aligned in the same manner as the unstained one-dimensional gel with the two-dimensional gel. The M_r of the cross-linked product in one-dimensional gels was determined from the mobility of standard proteins in parallel gels. Standards were as listed in the legend to Fig. 13 in Ref. 22. *XL*, cross-linked.

would have also been well separated in the first dimension and would have yielded diagonally related spots in two-dimensional gels.

The smear of p29 and p34 in the gels toward higher M_r in dimension one indicated that some p29,34 was aggregated. This aggregation was overcome in SDS at 21° (Fig. 2), indicating that aggregates are not held together as tightly as the $(p29)_1(p34)_1$ structure and are probably composed of multiple units of the latter structure.

Other proteins present in the preparations used in the experiments in Fig. 5 can also be seen to be cross-linked. p105, a protein present in Pool A, is cross-linked in a $(p105)_2$ structure (Fig. 5*A*). Disulfide-linked p105 dimers have been found in preparations purified in the absence of dithiothreitol (32). p 44 derived from a $(p44)_1(p12)_1$ cross-linked structure (32) can be seen in Fig. 5*C*.

DISCUSSION

The subunits of the *HLA*-linked B cell alloantigen, p29 and p34, remain noncovalently associated in the presence of SDS at room temperature. p29 and p34 also coelectrofocus under nondenaturing conditions, although they focus separately at pI values of 6.1 and 5.2, respectively, under denaturing conditions. The B cell antigen was not heated in the latter experiment, thus eliminating the possibility that p29 and p34 could be held together by a heat-labile covalent bond. The cross-linking experiments show that the subunits of B cell antigens (in the absence of SDS) are associated in a $(p29)_1(p34)_1$ structure. It therefore seems a reasonable conclusion that the complex of p29 and p34 with an apparent M_r of 55,000 in SDS has the same subunit stoichiometry. Together with experiments showing that p29 and p34 are not linked by disulfide bonds (12), the present experiments establish that in the $(p29)_1(p34)_1$ structure, the subunits are firmly, but noncovalently associated.

In this paper, advantage was taken of the surprising finding that p29 and p34 remain noncovalently associated in the presence of SDS by using preparative SDS-polyacrylamide gel electrophoresis as the final step in the purification of the B cell antigen. To our knowledge, this is the first use of this technique for purification of a multisubunit protein. After this procedure, the B cell antigen remained an active immunogen and inhibited both the rabbit anti-p23,34 serum which was produced and a human *HLA*-linked B cell alloantiserum. Thus the p29,34 complex must retain a conformation in SDS at room temperature similar to its native state, since antibodies made against it react with the native form of p29,34 on the surface of B lymphocytes, and p29,34 which had been treated with SDS can inhibit this reaction as well as the reaction with antisera produced by alloimmunization. Examples of other proteins which are stable after treatment with SDS are the C_{55}-isopren-

oid alcohol phosphokinase (33) and phospholipase A1 (34) which retain enzymatic activity, and the influenza virus hemagglutinin (35), which retains activity. p29,34 is also remarkably stable to heat, retaining full antigenic activity after ½ h at 57°. SDS treatment of *HLA*-A,B antigens at room temperature results in loss of antigenic activity (22) and dissociation, but subunit dissociation by anti-β_2-microglobulin serum results in retention of antigenic activity in the *HLA*-A,B heavy chain subunit (36).

Studies on the heat stability of p29,34 in SDS showed that dissociation of the p29,34 complex into p29 and p34 was quantitatively paralleled by loss in antigenic activity. Since the antigenic determinants of globular proteins are usually dependent upon conformation (37), the heating experiments suggest that subunit dissociation is accompanied by drastic conformational changes. These changes probably consist of an unfolding of the polypeptide chains into the extended rod conformation (38) and an increase in the amount of bound SDS. Heat-dissociated p29 and p34, which failed to inhibit rabbit anti-p29,34 serum, also failed to elicit antibodies when injected into rabbits. While antibodies have been elicited to actin after heating and preparative SDS-gel electrophoresis (39), proteins which have been denatured do not usually elicit antibodies which cross-react with their undenatured conformation (37).

As expected for an antiserum to a protein selectively expressed on B lymphocytes, anti-p29,34 serum is a highly specific B cell reagent, as shown by tests on B and T cell cultured cell lines and with peripheral B and T lymphocytes from 48 individuals. Its reactivity with B cells from almost all individuals tested indicates it recognizes largely heteroantigenic rather than alloantigenic determinants. The alloantigenic determinant is present on p29,34, however, as demonstrated by inhibition of Po antiserum.[3] An antiserum similar to anti-p29,34 has been prepared against the papain cleavage product of B cell alloantigens p23,30 (14, 15). p23,30 also carries the alloantigenic determinant. This serum, anti-p23,30, is also B cell-specific and precipitates from detergent-solubilized extracts polypeptides of M_r = 29,000 and 34,000. Purified p23,30 inhibits anti-p29,34 serum, and SDS gel-purified p29,34 inhibits anti-p23,30 serum (44). p29,34 when digested with papain yields p23,30.[3] It is thus clearly established that p29,34 and p23,30 are different forms of the *HLA*-linked B cell alloantigen, one derived in intact form by detergent solubilization of membranes and the other derived by papain cleavage of membranes. Detergent-solubilized and papain-solubilized *HLA*-linked B cell alloantigens have thus been isolated by quite different purification procedures, and shown to be related. This provides further support for the conclusion that these molecules are in fact the *HLA*-linked B cell alloantigen.

The many similarities between p29,34 and mouse and guinea pig Ia antigens have been summarized elsewhere (1, 12) and strongly suggest that these molecules are homologous. However, mouse Ia antigens are dissimilar from human p29,34 in that they seem to be relatively heat-labile, since Nonidet P-40-solubilized Ia antigens form a coagulate after incubation at 37° for 16 h (20). However, the temperature stability of murine Ia antigens has not been thoroughly studied over a range of temperatures, and the coagulation at 37° could be due to some factor other than heat. Heat treatment of both human (40) and murine (41) lymphocytes at 45° for 1 h abrogates their ability to stimulate in mixed lymphocyte culture, but not their ability to stimulate production of cell-mediated lympholysis effector cells, if a mixed lymphocyte culture stimulating cell is added to the culture. *HLA*-A,B antigens remain intact after this treatment. Since freeze-thawed cells, which presumably contain intact Ia antigens, do not stimulate in mixed lymphocyte culture (42), heat treatment may inactivate some physiological process in stimulator cells, rather than the antigens which stimulate in mixed lymphocyte culture. This could be tested serologically on heat-treated cells.

Anti-p23,30 serum has been used to study the expression of the human Ia-like antigens on leukemia cells (43). Anti-p29,34 serum also has a similar pattern of reactivity with different human leukemias.[4] Since p29,34 can be readily obtained in highly pure form by preparative SDS-gel electrophoresis, it may thus be an excellent source of antigen for the production of antisera to the *HLA*-linked B cell-specific antigen.

[3] A. DeFranco, unpublished data.

[4] L. Chess, unpublished data.

REFERENCES

1. Bach, F. H., Bach, M. L., and Sondel, P. M. (1976) *Nature* **259**, 273–281
2. Mann, D. L., Abelson, L., Harris, S., and Amos, D. B. (1976) *Nature* **259**, 145–146
3. Mann, D. L., Abelson, L., Henkart, P., Hanis, S. D., and Amos, D. B. (1975) *Proc. Natl. Acad. Sci. U. S. A.* **72**, 5103–5106
4. van Rood, J. J., van Leeuwen, A., Keuning, J. J., Blussé, and van Oud Albas, A. (1975) *Tissue Antigens* **5**, 73–79
5. Winchester, R. J., Fu, S. M., Wernet, P., Kunkel, H. G., Dupont, B., and Jersild, C. (1975) *J. Exp. Med.* **141**, 924–929
6. Solheim, B. G., Bratlic, A., Winther, N., and Thorsby, E. (1975) in *Histocompatibility Testing* (Kissmeyer-Nielsen, F., ed) pp. 713–718, Munksgaard, Copenhagen
7. Kovithavongs, T., Hyskha, L., MaConnachie, P. R., and Dossetor, J. B. (1975) *Tissue Antigens* **5**, 165–172
8. Mann, D. L., Abelson, L., Harris, S., and Amos, D. B. (1975) *J. Exp. Med.* **142**, 84–89
9. Walford, R. L., Gossett, T., Smith, G. S., Zeller, E., and Wilkinson, J. (1975) *Tissue Antigens* **5**, 196–204
10. Legrand, L., and Dausset, F. (1975) in *Histocompatibility Testing* (Kissmeyer-Nielsen, F., ed) pp. 665–670, Munksgaard, Copenhagen
11. Jones, E. A., Goodfellow, P. N., Bodmer, J. G., and Bodmer, W. F. (1975) *Nature* **256**, 650–652
12. Springer, T., Kaufman, J., Terhorst, C., and Strominger, J. L. (1977) *Nature*, in press
13. Bondevik, H., Albrechtsen, D., Solheim, B. G., and Thorsby, E. (1975) in *Histocompatibility Testing* (Kissmeyer-Nielsen, F., ed) pp. 604–607, Munksgaard, Copenhagen
14. Strominger, J. L., Chess, L., Herrmann, H. C., Humphreys, R. E., Malenka, D., Mann, D., McCune, J. M., Parham, P., Robb, R., Springer, T. A., and Terhorst, C. (1975) in *Histocompatibility Testing* (Kissmeyer-Nielsen, F., ed) pp. 719–730, Munksgaard, Copenhagen
15. Humphreys, R. E., McCune, J. M., Chess, L., Herrman, H. C., Malenka, D. J., Mann, D. L., Parham, P., Schlossman, S. F., and Strominger, J. L. (1976) *J. Exp. Med.* **144**, 98–112
16. Cresswell, P., and Geier, S. S. (1975) *Nature* **257**, 147–149
17. Wernet, P., and Kunkel, H. G. (1975) in *Histocompatibility Testing* (Kissmeyer-Nielsen, F., ed) pp. 731–734, Munksgaard, Copenhagen
18. Shreffler, D. C., and David, C. S. (1975) *Adv. Immunol.* **20**, 125–195
19. Cullen, S. E., David, C. S., Cone, J. L., and Sachs, D. H. (1976) *J. Immunol.* **116**, 549–553
20. Cullen, S. E., David, C. S., Shreffler, D. C., and Nathenson, S. G. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 648–652
21. Springer, T. A., Strominger, J. L., and Mann, D. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 1539–1543
22. Springer, T. A., Mann, D. L., DeFranco, A. L., and Strominger, J. L. (1977) *J. Biol. Chem.* **252**, 4682–4693
23. Springer, T. A., and Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 2481–2485

24. Vesterberg, O. (1971) *Biochim. Biophys. Acta* **243**, 345–348
25. Studier, F. W. (1973) *J. Mol. Biol.* **79**, 230–248
26. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
27. David, C., Meo, T., McCormick, J., and Shreffler, D. (1976) *J. Exp. Med.* **143**, 218–224
28. Springer, T. A., Kaufman, J. F., Siddoway, L. A., Giphart, M., Mann, D. L., Terhorst, C., and Strominger, J. L. (1977) *Cold Spring Harbor Symp. Quan. Biol.* **41**, 387–396
29. Parham, P., Humphreys, R. E., Turner, M. J., and Strominger, J. L. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 3998–4001
30. Wang, K., and Richards, F. M. (1974) *J. Biol. Chem.* **249**, 8005–8018
31. Lomant, A. J., and Fairbanks, G. (1976) *J. Mol. Biol.* **104**, 243
32. Springer, T. A., Robb, R. J., Terhorst, C. and Strominger, J. L. (1977) *J. Biol. Chem.* **252**, 4694–4700
33. Sandermann, H., and Strominger, J. L. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 2441–2443
34. Scandella, C. J., and Kornberg, A. (1971) *Biochemistry* **10**, 4447–4456
35. Laver, W. G. (1963) *Virology* **20**, 251–262
36. Nakamuro, K., Tanigaki, N., and Pressman, D. (1975) *Transplantation* **19**, 431–437
37. Arnon, R. (1973) in *The Antigens* (Sela, M., ed) Vol. 1, p. 110, Academic Press, New York
38. Reynolds, J. A., and Tanford, C. (1970) *J. Biol. Chem.* **245**, 5161–5165
39. Lazarides, E., and Weber, K. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 2268–2272
40. Eijsvoogel, V. P., duBois, M., Meiniesz, A., Bierhorst-Eijlander, A., Zeylemaker, W., and Schellekens, P. (1973) *Transplant. Proc.* **5**, 1675–1678
41. Schendel, D. J., and Bach, F. H. (1974) *J. Exp. Med.* **140**, 1534–1546
42. Ling, N. R., and Kay, J. E. (1975) *Lymphocyte Stimulation*, pp. 133–134, North-Holland, Amsterdam
43. Schlossman, S. F., Chess, L., Humphreys, R. E., and Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 1288–1291
44. Kaufman, J., Fuks, A., Giphart, M., Solheim, B., and Strominger, J. L. (1977) *Proceedings of the Third Ir Gene Workshop, Asilomar, December 12–16, 1976* (McDevitt, H., ed) Academic Press, New York, in press