Proc. Natl. Acad. Sci. USA
Vol. 75, No. 12, pp. 6002–6006, December 1978
Biochemistry

Phosphorylation *in vivo* and *in vitro* of human histocompatibility antigens (HLA-A and HLA-B) in the carboxy-terminal intracellular domain

(membrane proteins/protein kinase/cytoskeleton/lymphocyte activation)

JORDAN S. POBER, BRAYDON C. GUILD, AND JACK L. STROMINGER

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Contributed by Jack L. Strominger, October 3, 1978

ABSTRACT **HLA-A and -B antigens are phosphorylated in transformed lymphoblastoid cells and peripheral blood lymphocytes, both incubated with $^{32}P_i$. The phosphate group is attached to HLA-A and -B heavy chain (p44) as identified by immunoprecipitation with anti-β_2-microglobulin IgG, sodium dodecyl sulfate/polyacrylamide gel electrophoresis, isoelectric focusing, and susceptibility to limited proteolysis by papain and trypsin. The site(s) of phosphorylation is identified as a serine residue(s) located in the hydrophilic carboxy terminus of the p44 chain. HLA antigens are also phosphorylated in isolated membranes from transformed lymphoblastoid cells that are incubated with [γ^{32}P]ATP. The phosphorylation of the carboxy terminus of HLA-A and -B antigens *in vivo* is good evidence that this portion of the molecule is intracellular. Furthermore, this modification suggests a general way in which interactions between membrane proteins and cytoskeletal elements may be regulated.**

The means by which cells arrange their surface proteins is a fundamental problem in cell biology and is believed to involve interactions between membrane proteins and cytoplasmic elements (1, 2). Histocompatibility antigens are cell surface proteins whose movement in the plasma membrane appears to be coordinated with the movement of intracellular cytoskeletal elements (3, 4), suggesting an association. More is known about the structure of human histocompatibility antigens (HLA-A and -B) than about the structure of any other plasma membrane protein of a nucleated cell, so these molecules offer the best opportunity to study the structural basis of putative membrane protein–cytoplasmic protein interactions. Recently evidence has been presented for a direct association between mouse histocompatibility antigens (H2-K and -D) and actin (5). Similar associations involving HLA-A and -B antigens may also occur.

HLA-A and -B antigens, when purified in detergent solutions, are composed of a 44,000-dalton glycoprotein heavy chain (p44) and a 12,000-dalton light chain (p12), β_2-microglobulin. A complex between the amino terminus of the p44 chain and the p12 chain forms an extracellular domain that may be liberated by papain from the cell surface as a p34,12 soluble antigen (6). The carboxy terminus of the p44 chain contains both a hydrophilic stretch of 32 amino acid residues and a penultimate sequence of 25 amino acid residues that is markedly hydrophobic (7, 8). It has been proposed that the hydrophobic sequence of HLA-A and -B spans the lipid bilayer of the membrane and that the hydrophilic carboxy-terminal sequence is located intracellularly (7, 8). A similar structure has been demonstrated for human glycophorin A (9, 10). This intracellular carboxy-terminal domain of HLA-A and -B would thus be able to interact with cytoplasmic elements. Walsh and Crumpton (11) have presented evidence that HLA-A and -B antigens do span the plasma membrane, but they did not identify the intracellular portion of the chain.

Recently, Robb *et al.* (8) have determined the amino acid sequence of the hydrophilic carboxy terminus of the p44 chains of HLA-B7 and -A2. In HLA-B7, the carboxy terminus contains 32 amino acid residues, 8 of which are serine and 1 of which is threonine. HLA-A2 appears to contain an additional serine. Phosphorylation of serine or threonine hydroxyl groups by specific protein kinases is a well known method of regulating enzyme function (12). Lymphocytes alter their level of protein phosphorylation upon mitogenic stimulation (13), though the protein targets have not been identified. Membrane protein phosphorylation has been shown to mediate membrane permeability in neural and other tissues (12, 14). Glycophorin A, which has a transmembrane arrangement similar to that proposed for HLA-A and -B antigens, is phosphorylated in its intracellular carboxy terminal region, though only to a small extent (15). Most interesting are the observations that a number of proteins that interact with actin, including myosin (16), troponin (17, 18), tropomyosin (19), filamin (20), and spectrin (21), are phosphorylated. In skeletal muscle, myosin phosphorylation fluctuates with muscle contraction (22, 23), and in smooth muscle and nonmuscle cells, myosin phosphorylation alters actomyosin ATPase (24). Recently, it has been shown that spectrin phosphorylation induces actin gel formation *in vitro* (25), providing evidence that phosphorylation could play a role in controlling cytoskeletal protein interactions. Phosphorylation of the carboxy terminus of HLA-A and -B is thus a potential means of regulating associations between the intracellular domain of this membrane protein and cytoskeletal proteins. To this end, we have begun an investigation of phosphorylation in lymphoid cells and report here that the carboxy terminus of HLA-A and -B antigens is phosphorylated *in vivo* and *in vitro*.

MATERIALS AND METHODS

Materials. JY transformed lymphoblastoid cells (homozygous HLA-A2 and -B7) were used in most experiments. Peripheral blood lymphocytes were obtained from a volunteer donor. Rabbit anti-β_2-microglobulin IgG-Sepharose beads were synthesized by Richard Robb. Detergent-purified HLA-A and -B antigens, a mixture of HLA-A2 and -B7, were prepared from JY cell membranes as described (26). Papain (13 units/mg) and *N*-tosylphenylalanine chloromethyl ketone (TPCK)-treated trypsin (246 units/mg) were obtained from Worthington. Nonidet P-40 (NP-40) was obtained from Particle Data Laboratories, Elmhurst, IL. All reagents for sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: NP-40, Nonidet P-40; $NaDodSO_4$, sodium dodecyl sulfate.

from Bio-Rad. Ampholines for isoelectric focusing were from LKB. Sodium $^{32}PO_4$ (1 Ci/mmol), $H_3{}^{32}PO_4$ (carrier-free), and [γ-^{32}P]ATP (3000 Ci/mmol) were obtained from New England Nuclear. All other chemicals were reagent grade.

Labeling of Cells. JY cells (2×10^7) were washed free of medium in phosphate-free buffer A (150 mM NaCl/5 mM $MgCl_2$/5 mM KCl/2 mM glutamine/1.8 mM glucose/10 mM Tris-acetate, pH 7.4) and incubated in buffer A for 1 hr at 37°C. The cells were then resuspended in 10 ml of buffer A containing 10 mCi of $^{32}P_i$ either at 1 Ci/mmol or carrier-free, and incubated for an additional 3 hr at 37°C. The cells were then brought to ice temperature, pelleted, and washed three times with phosphate-containing buffer B (137 mM NaCl/2.6 mM KCl/1.4 mM potassium phosphate and 8.6 mM sodium phosphate, pH 7.2). The final pellet was lysed at 0°C in 2% NP-40 in buffer C (25 mM NaCl/5 mM $MgCl_2$/25 mM Tris·HCl, pH 7.4) made 1 mM in phenylmethanesulfonyl fluoride. This detergent lysate was Vortex mixed intermittently for 15 min and then spun at 17,000 rpm for 20 min in a Sorvall SS34 rotor. The clear supernatant is designated as the NP-40 extract. In some experiments, peripheral blood lymphocytes were prepared by Ficoll/Hypaque flotation and used in place of JY cells.

Immunoprecipitation of HLA-A and -B. Rabbit anti-human β_2-microglobulin IgG coupled to Sepharose beads was equilibrated for use by centrifugation three times in buffer C containing 2% NP-40, 1 mM phenylmethanesulfonyl fluoride, and 0.3% bovine serum albumin. NP-40 extract (1 ml) was added to 0.1 ml of packed resin and the suspension was continuously agitated on a modified Vortex mixer for 1–2 hr. (In some experiments, the NP-40 extract was preincubated with rabbit anti-goat Ig coupled to Sepharose beads for 30 min to eliminate nonspecific adsorption; this step did not change the results.) After adsorption, the supernatant was removed and the beads were washed two or three times in buffer C containing 2% NP-40, 1 mM phenylmethanesulfonyl fluoride, and 0.3% bovine serum albumin and then washed with 0.2% NP-40 in buffer E (10 mM Tris·HCl, pH 7.4) five to seven times until the radioactivity of the supernatant decreased to less than 1000 cpm total.

Elution of native HLA-A and -B was achieved by incubation of the beads with 2 vol of β_2-microglobulin at 500 μg/ml in 0.2% NP-40/buffer E for 36 hr followed by washing with 3 vol of 0.2% NP-40/buffer E. If native HLA-A and -B was not required, the beads were eluted either with 10% acetic acid or 1% $NaDodSO_4$.

Labeling of Membranes. Membranes were prepared from JY cells by nitrogen cavitation (27) and either were used immediately or stored frozen at −70°C. Prior to labeling, the membranes were washed twice in buffer D (140 mM NaCl/10 mM Tris·HCl, pH 7.4/5 mM $MgCl_2$). The final pellet was suspended in this buffer at a concentration of membranes equal to 1×10^7 cells per ml. This solution (1 ml) was made 1 mM in ATP containing 1000 mCi of [γ-^{32}P]ATP per mmol and incubated for 1 hr at 37°C. The reaction was stopped by washing the membranes three times in buffer B and the pellet was extracted with NP-40 as in whole cells.

Limited Proteolysis of HLA-A and -B. Twenty-five microliters of ^{32}P-labeled native HLA-A and -B, eluted from the anti-β_2-microglobulin IgG-Sepharose with excess β_2-microglobulin as above, was mixed with 50 μl containing 15 μg of cold carrier HLA-A and -B in 0.1% deoxycholate/10 mM Tris·HCl, pH 7.4. Trypsin was added in a total volume of 25 μl of buffer E at a final weight ratio to HLA-A and -B of 1/1000, 1/200, or 1/40. Papain was added in a total volume of 25 μl of buffer E containing 2 mM dithiothreitol and 2 mM EDTA at a final weight ratio to HLA-A and -B of 1/5000, 1/1000, or 1/200. Samples were incubated with protease for 1 hr at 37°C. The reaction was stopped by addition of 5 mM inhibitor and incubated for 20 min at room temperature. The inhibitors used were phenylmethanesulfonyl fluoride for trypsin and iodoacetic acid for papain. The samples were then precipitated by the addition of 600 μl of acetone, mixed thoroughly, and kept at −20°C for a minimum of 2 hr. Just prior to $NaDodSO_4$/polyacrylamide gel electrophoresis, the acetone solutions were centrifuged at 6000 rpm for 15 min. Greater than 95% of the clear supernatant was aspirated off, and the residue was dried under a nitrogen stream. This method has been shown to give quantitative precipitation of HLA-A and -B antigens (R. Robb and J. L. Strominger, unpublished data). Similar results were obtained when the proteolysis samples were lyophilized instead of acetone-precipitated.

Analytic Methods. $NaDodSO_4$/polyacrylamide gel electrophoresis was performed according to the method of Laemmli (28) on a slab gel. The gels were fixed and stained by method A of Vesterberg and Hansen (29). Autoradiography was performed on wet or dried gels by using Kodak XR5 or SB5 x-ray film, with and without lightning screens. Isoelectric focusing was performed on a flat bed acrylamide slab containing 6 M urea and 2.0% NP-40 (8). The pH gradient was 4–9.

Phosphorylated amino acid residues were identified by 6 M HCl hydrolysis at reduced pressure at 100°C for 2 hr and subsequent high voltage paper electrophoresis in 2.1% formic acid/8.7% acetic acid (15). These conditions cleanly resolved phosphoserine from phosphothreonine.

RESULTS

HLA-A and -B Antigens are Phosphorylated in Cultured Lymphoblastoid Cells. When JY transformed lymphoblastoid cells were incubated with $^{32}P_i$, many cell proteins were phosphorylated as shown by $NaDodSO_4$/polyacrylamide gel electrophoresis of a 2% NP-40 extract (Fig. 1). Under these conditions, phosphorylation of HLA-A and -B antigens probably

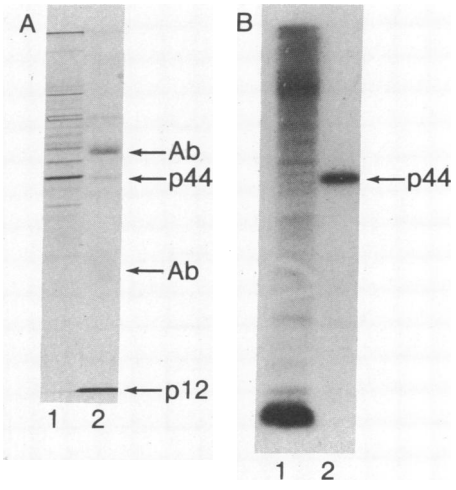

FIG. 1. (*A*) Coomassie blue staining pattern of $NaDodSO_4$/polyacrylamide gel showing in lane 1 all the proteins of transformed lymphoblastoid cells and in lane 2 those proteins immunoprecipitated by anti-β_2-microglobulin IgG. The immunoprecipitate is concentrated about 50-fold relative to the original extract and contains antibody heavy and light chains (Ab) as well as p44 and p12 from HLA -A and -B antigens. (*B*) Autoradiograph of ^{32}P incorporated into proteins on the same gels as in *A*. Lane 1 shows multiple phosphorylated proteins, whereas lane 2 shows that immunoprecipitation by beads containing anti-β_2-microglobulin IgG results in the isolation of one major phosphorylated species which migrates at the position of p44. Note that β_2-microglobulin is not labeled. (The magnifications in *A* and *B* are slightly different.)

represents less than 1% of total protein phosphorylation. Nevertheless, when the 2% NP-40 extract was immunoprecipitated with rabbit anti-human β_2-microglobulin IgG coupled to Sepharose beads, the washed immunoprecipitate contained only one major radioactive species, as detected by autoradiography of $NaDodSO_4$/polyacrylamide gel. This ^{32}P-labeled phosphoprotein comigrated with purified detergent-soluble HLA-A and -B heavy chain (p44). No radioactivity was seen to comigrate with β_2-microglobulin (p12). Actin, which migrates similarly to p44, was clearly resolved from the radioactive band on some gels (not shown).

The identification of the immunoprecipitated radioactive phosphoprotein as HLA-A and -B heavy chain is confirmed by two additional lines of evidence. First, JY cells contain both HLA-A2 and -B7 antigens. The heavy chains of the detergent soluble form of these two antigens (p44) characteristically show different patterns upon isoelectric focusing in NP-40 and urea: p44 from HLA-A2 produces a cluster of bands in the same region of the pH gradient as β_2-microglobulin (approximately pH 6.5), whereas p44 from HLA-B7 focuses as a cluster of bands at markedly lower pH (approximately pH 5.0) (8). The immunoprecipitated ^{32}P-labeled phosphoprotein focused as three major bands in the region of carrier HLA-A2 p44 and as three major bands in the region of carrier HLA-B7 p44 (Fig. 2). *The immunoprecipitated phosphoprotein exhibited the characteristic separation of HLA-A2 and -B7 heavy chains.* Incidentally, the multiple bands seen upon focusing p44 from detergent-purified HLA-A and -B were attributed initially solely to differences in sialic acid content (8) as was shown for p34 from papain-solubilized HLA-A and -B (30). Our results indicate that part of the micro-heterogeneity of detergent-purified p44 may be due to differential phosphorylation.

In addition to the isoelectric focusing data, the identification of the radioactive phosphoprotein as HLA-A and -B heavy chain was also shown by the susceptibility of the immunoprecipitated phosphoprotein to the action of proteases. In this experiment, immunoprecipitated ^{32}P-labeled phosphoprotein was eluted from the anti-β_2-microglobulin IgG beads by incubation with excess β_2-microglobulin. The eluate was mixed with detergent-purified carrier HLA-A2 and -B7, the mixture was subjected to limited proteolysis by trypsin or by papain, and the results were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. As seen in Fig. 3, radioactivity disappeared from the position of HLA-A and -B heavy chain (p44) at the same rate as this chain was cleaved (as detected by Coomassie blue staining). *Thus, the immunoprecipitated phosphoprotein has precisely the same susceptibility to trypsin and to papain as HLA-A and -B antigens.* Taken together with the isoelectric focusing data, the identity of the phosphoprotein as HLA-A and -B antigen is established.

The Site of Phosphorylation in the Carboxy Terminus. The limited proteolysis experiment described above also served to locate the site of phosphorylation. As the weight ratio of trypsin to HLA-A and -B antigen was increased, the heavy chain decreased from an apparent molecular weight of 44,000 to 39,000 (Fig. 3). No radioactivity was associated with the 39,000-dalton species. Similarly, as the ratio of papain to HLA-A and -B antigen was increased, the heavy chain decreased from 44,000 to 39,000 to 34,000 daltons. No radioactivity was associated with either the 39,000- or 34,000-dalton chains (Fig. 3). Papain is

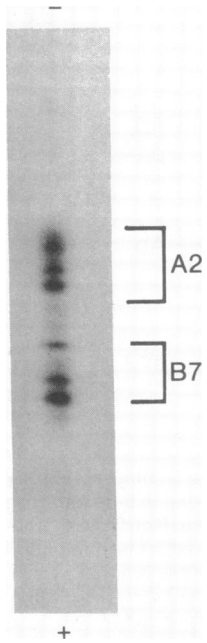

FIG. 2. Autoradiograph of an isoelectric focusing gel in 2.0% NP-40 and 6 M urea, pH 4–9. The major ^{32}P phosphorylated species cluster into two sets of bands that correspond to the position of several of the major Coomassie blue stained bands of HLA-A2 and HLA-B7.

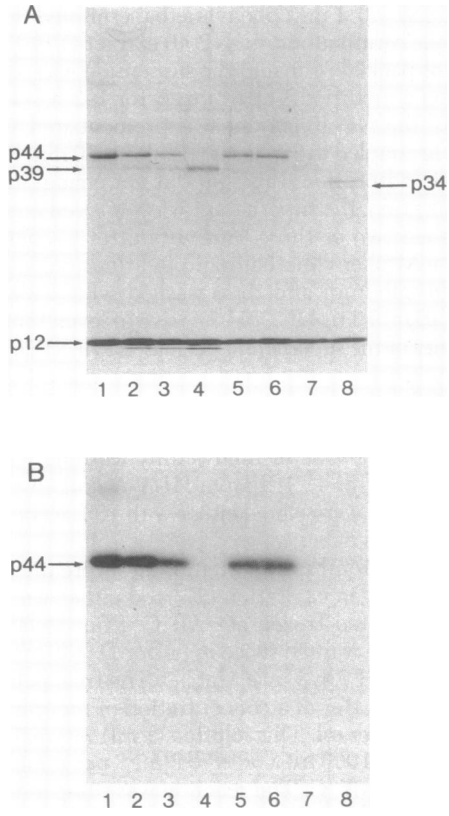

FIG. 3. (*A*) Coomassie blue staining pattern after limited proteolysis of native HLA-A and -B (i.e., HLA-A and -B isolated by elution from an anti-β_2-microglobulin IgG affinity column with excess free β_2-microglobulin). Lanes 1–4 represent weight ratios of trypsin to HLA-A and -B of 0, 1:1000, 1:200 and 1:40, respectively; lanes 5–8 represent weight ratios of papain to HLA-A and -B of 0, 1:5000, 1:1000 and 1:200, respectively, as described in the text. Trypsin produced conversion of p44 to p39; papain produced conversion of p44 to p39 and then to p34. Note that p34 exists as a doublet corresponding to the separation of HLA-A2 and -B7 seen on Laemmli gels. (*B*) Autoradiograph of the same gel as in *A* revealing ^{32}P-labeled phosphoproteins. Note that radioactivity was lost at the same rate that p44 disappeared in *A* and that neither p39 nor p34 contained radioactivity.

known to act on detergent soluble HLA-A and -B by progressively liberating peptides from the carboxy terminus of the heavy chain (7). Trypsin is also believed to release a carboxy-terminal peptide(s) (31). Because both proteases liberate the bound phosphate as they release carboxy-terminal peptides, it follows that the phosphate groups must be attached to the carboxy-terminal region of the polypeptide chain. High voltage paper electrophoresis subsequent to limited acid hydrolysis revealed that the phosphorylated residue was serine, though small amounts of phosphothreonine below the level of detection are not excluded. It is of interest that the sequence of the carboxy-terminal hydrophilic region of HLA-B7 contains 8 serine residues in a stretch of 32 residues. The precise serine(s) modified has not yet been determined.

HLA-A and -B Antigens are Phosphorylated in Peripheral Blood Lymphocytes. Peripheral blood lymphocytes contain only about 10% as much HLA-A and -B antigens on their cell surface as the transformed lymphoblastoid cell lines used above (32). Nevertheless, the same procedures of NP-40 extraction and immunoprecipitation with anti-β_2-microglobulin IgG-Sepharose beads resulted in the isolation of small quantities of protein that comigrated with purified HLA-A and -B on NaDodSO$_4$/polyacrylamide gels (Fig. 4*A*). When peripheral blood lymphocytes were incubated with $^{32}P_i$ for 1 hr, many cell proteins were phosphorylated (Fig. 4*B*). As with transformed lymphoblastoid cells, only one ^{32}P-labeled phosphoprotein was immunoprecipitated by the anti-β_2-microglobulin IgG-Sepharose beads. This protein corresponded to the Coomassie blue-stained band identified above as HLA-A and -B heavy chain (p44). Thus, peripheral blood lymphocytes phosphorylate their HLA-A and -B antigens *in vivo*.

Lymphocyte Membranes Catalyze the Phosphorylation of HLA-A and -B Antigens. Isolated membranes from JY lymphoblastoid cells catalyze the phosphorylation of HLA-A and -B antigens when incubated with [γ-^{32}P]ATP as revealed by immunoprecipitation and autoradiography (Fig. 5). The reaction appears to be less efficient than in whole cells. Incubation of membranes with $^{32}P_i$ did not produce detectable phosphorylation of HLA-A and -B antigens by autoradiography. The phosphorylation with [γ-^{32}P]ATP could be blocked by boiling membranes for 3 min, providing evidence that the phosphorylation is enzyme catalyzed. Because the cell sap had been removed by repeated centrifugal washes, a part of the protein kinase activity is probably membrane bound. These data do not exclude the possibility that additional kinases are present in the cytoplasm. When HLA-A and -B antigens were phosphorylated *in vitro*, and then were subjected to limited trypsin treatment in detergent solution, the label was lost as p44 was cleaved to p39. Thus, as in intact lymphocytes, the site(s) of phosphorylation appears to be specific and is located in the carboxy terminus of the p44 chain.

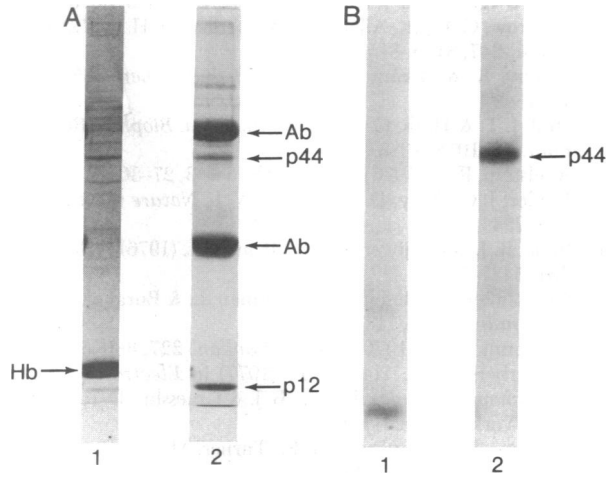

FIG. 4. (*A*) Coomassie blue staining pattern of NaDodSO$_4$/polyacrylamide gels showing in gel 1 all of the proteins of peripheral blood lymphocytes and in gel 2 proteins immunoprecipitated by beads containing anti-β_2-microglobulin IgG. The peripheralblood lymphocytes were contaminated by erythrocytes as revealed by the hemoglobin (Hb) doublet near the dye front in gel 1. As with transformed lymphoblastoid cells, the anti-β_2-microglobulin IgG immunoprecipitate was concentrated about 50-fold relative to the original extract and contained antibody heavy and light chains (Ab), as well as p44 and p12. (*B*) Autoradiograph of gels in *A*. As in transformed lymphoblastoid cells, peripheral blood lymphocytes show many phosphorylated proteins, but the anti-β_2-microglobulin IgG immunoprecipitation selectively purified one ^{32}P-labeled phosphoprotein which migrated at the position of p44. The autoradiograph pattern in gel 1 is faint in the reproduction.

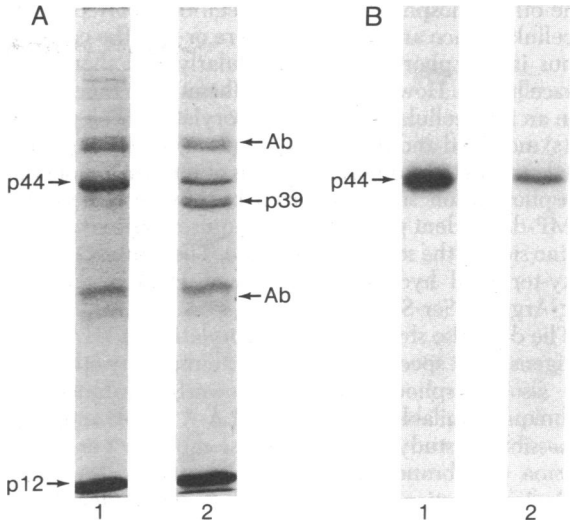

FIG. 5. (*A*) Coomassie blue staining pattern of native HLA-A and -B antigens purified from isolated membranes of lymphoblastoid cells by detergent solubilization and immunoprecipitation. The isolated membranes were incubated with [γ^{32}P]ATP prior to solubilization. Gel 1 shows p44, 12 in untreated membranes, whereas gel 2 shows partial conversion of p44 to p39 after limited proteolysis with trypsin. (*B*) Autoradiograph of the same gels as in (*A*) showing incorporation of $^{32}PO_4^{3-}$ into p44 (but not p12) after incubation of isolated membranes with [γ^{32}P]ATP and loss of $^{32}PO_4^{3-}$ from p44 concomitant with its conversion to p39 by trypsin.

DISCUSSION

The data presented in this paper show that HLA-A and -B antigens are phosphorylated *in vivo* by lymphoblastoid cells. The site(s) of phosphorylation has been identified as a serine residue(s) and has been localized by limited proteolysis with papain and with trypsin to the carboxy terminus of the p44 chain. The facts that the label comigrates with p44 on NaDodSO$_4$/polyacrylamide gels, that the label is associated with a hydrophilic portion of the protein, and that phosphoserine has been identified exclude the possibility that the phosphate is in lipid that is associated with the protein. The label was presented to the cells as P_i and it is reasonable to assume that the labeling proceeds via a high energy phosphorylated intermediate (e.g., ATP). Because ATP is synthesized intracellularly, the specific phosphorylation of the carboxy terminus of p44 is good evidence that this portion of the chain is intracellular. Obviously, the data presented do not exclude the possibility that ATP or

some other phosphate donor has been transported to the extracellular space and is utilized there or that the carboxy terminus is phosphorylated intracellularly and then extruded extracellularly. However these hypotheses seem much less likely than an intracellular site of phosphorylation. The precise serine(s) modified and the number of phosphate groups incorporated remain to be determined. It is of interest to note that the phosphorylation site recognized by bovine skeletal muscle cAMP-dependent protein kinase requires basic residues to the amino side of the serine residue (33). The sequence of the carboxy-terminal hydrophilic region of HLA-B7 begins Cys-Arg-Arg-Lys-Ser-Ser- (8).

The data also show that phosphorylation of HLA-A and -B antigens is not specific for these proteins; many other proteins are also phosphorylated. The powerful immunochemical techniques available for isolating HLA-A and -B antigens make it possible to study selectively these molecules as a model of plasma membrane protein phosphorylation. An interesting biological question is how membrane protein phosphorylation is regulated. Parker (13) has shown that mitogenic stimulation of peripheral blood lymphocytes produces a transient burst of protein phosphorylation. Peripheral blood lymphocytes also phosphorylate their HLA-A and -B antigens. It would be of interest to determine whether the phosphorylation of HLA-A and -B antigens fluctuates with lymphocyte activation.

It is interesting to speculate that stimuli that alter the membrane topology could do so by influencing a protein kinase or phosphatase. According to this hypothesis, membrane protein phosphorylation would be able to regulate the arrangement of cell surface proteins by modulating membrane protein–cytoskeletal interactions. Various proteins that bind to actin have been shown to be phosphorylated (16–21). Most interesting has been data that show that phosphorylation of nonmuscle and smooth muscle myosin changes the activity of actomyosin ATPase (24) and that phosphorylation of spectrin changes its interaction with actin *in vitro* (25). Mouse histocompatibility antigens have been shown to interact with actin (5); it would be of great interest to know whether HLA-A and -B antigens also bind to actin and whether phosphorylation of the carboxy terminus could alter this interaction. If so, the phosphorylation of HLA-A and -B antigens could provide experimental access to the structural basis of membrane protein–cytoskeletal interactions.

Note Added in Proof. Microphosphate analyses, kindly carried out by O. H. Lowry, on a sample of the heavy chain of HLA-B7 purified from JY lymphoblastoid cells (8) revealed the presence of 0.7 mol of organic phosphate per mol of protein (quantitated by amino acid analysis). If, as seems likely, the total organic phosphate of HLA-B7 is comprised of only the serine phosphate moiety detected by autoradiography, then a high proportion of the HLA-A and -B antigens are phosphorylated in their carboxy termini in this transformed lymphoblastoid cell line.

This work was supported by a research grant from the National Institutes of Health (AI 10736). J.S.P. is a postdoctoral fellow of the Anna Fuller Fund.

1. Nicolson, G. L. (1976) *Biochim. Biophys. Acta* **457**, 57–108.
2. Edelman, G. M. (1976) *Science* **192**, 218–226.
3. Bourguignon, L. Y. W. & Singer, S. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5031–5035.
4. Ash, J. F., Louvard, D. & Singer, S. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5584–5588.
5. Koch, G. L. E. & Smith, M. J. (1978) *Nature (London)* **273**, 274–278.
6. Cresswell, P., Turner, M. & Strominger, J. L. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 1603–1607.
7. Springer, T. & Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2481–2485.
8. Robb, R. J., Terhorst, C. & Strominger, J. L. (1978) *J. Biol. Chem.* **253**, 5319–5324.
9. Tomita, M. & Marchesi, V. T. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2964–2968.
10. Cotmore, S. F., Furthmayr, H. & Marchesi, V. T. (1977) *J. Mol. Biol.* **113**, 539–553.
11. Walsh, F. S. & Crumpton, M. J. (1977) *Nature (London)* **269**, 307–311.
12. Rubin, C. S. & Rosen, O. M. (1975) *Annu. Rev. Biochem.* **44**, 831–887.
13. Parker, C. W. (1978) in *Advances in Cyclic Nucleotide Research*, eds. George, W. J. & Ignarro, L. S. (Raven, New York), Vol. 9, pp. 647–660.
14. Greengard, P. (1975) in *Advances in Cyclic Nucleotide Research*, eds. Drummond, G. I., Greengard, P. & Robison, G. A. (Raven, New York), Vol. 5, pp. 585–601.
15. Shapiro, D. L. & Marchesi, V. T. (1977) *J. Biol. Chem.* **252**, 508–517.
16. Perrie, W. T., Smillie, L. B. & Perry, S. V. (1972) *Biochem. J.* **128**, 105P–106P.
17. Stull, J. T., Brostrom, C. O. & Krebs, E. G. (1972) *J. Biol. Chem.* **247**, 5272–5274.
18. England, P. J., Stull, J. T. & Krebs, E. G. (1972) *J. Biol. Chem.* **247**, 5275–5277.
19. Ribolow, H. & Bárány, M. (1977) *Arch. Biochem. Biophys.* **179**, 718–720.
20. Wallach, D., Davies, P., Bechtel, P., Willingham, M. & Pastan, I. (1978) in *Advances in Cyclic Nucleotide Research*, eds. George, W. J. & Ignarro, L. J. (Raven, New York), Vol. 9, pp. 371–379.
21. Guthrow, C. E., Jr., Allen, J. E. & Rasmussen, H. (1972) *J. Biol. Chem.* **247**, 8145–8153.
22. Bárány, K. & Bárány, M. (1977). *J. Biol. Chem.* **252**, 4752–4754.
23. Stull, J. T. & High, C. W. (1977) *Biochem. Biophys. Res. Commun.* **77**, 1078–1083.
24. Adelstein, R. S. (1978) *Trends Biol. Sci.* **3**, 27–30.
25. Pinder, J. C., Bray, D. & Gratzer, W. B. *Nature (London)* **270**, 752–754.
26. Robb, R. J., Strominger, J. L. & Mann, D. L. (1976) *J. Biol. Chem.* **251**, 5427–5428.
27. Lemonnier, F., Mescher, M., Sherman, L. & Burakoff, S. (1978) *J. Immunol.* **120**, 1114–1120.
28. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
29. Vesterberg, O. & Hansén, L. (1977) in *Electrofocusing and Isotachophoresis*, eds. Radola, B. J. & Graesslin, D. (de Gruyter, New York), pp. 123–133.
30. Parham, P., Humphreys, R. E., Turner, M. J. & Strominger, J. L. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3998–4001.
31. Engelhard, V. H., Guild, B. C., Helenius, A., Terhorst, C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3230–3234.
32. McCune, M. M., Humphreys, R. E., Yocum, R. R. & Strominger, J. L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3206–3209.
33. Kemp, B. E., Graves, D. J., Benjamini, E. & Krebs, E. G. (1977) *J. Biol. Chem.* **252**, 4888–4894.