Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4759–4763, July 1986
Cell Biology

Phosphorylation of the rat hepatic polymeric IgA receptor

(total microsomes/bile/ectodomain/endodomain/phosphoserine)

JANET M. LARKIN*, ELIZABETH S. SZTUL†, AND GEORGE E. PALADE*

Departments of *Cell Biology and †Human Genetics, Yale University School of Medicine, 333 Cedar Street; P. O. Box 3333, New Haven, CT 06510

Contributed by George E. Palade, March 3, 1986

ABSTRACT **_In vivo_ labeling with [^{35}S]cysteine has identified three transmembrane forms of the rat hepatic polymeric IgA receptor: (*i*) a 105-kDa core glycosylated precursor; (*ii*) a terminally glycosylated 116-kDa intermediate; and (*iii*) a mature 120-kDa form. In the current study we show that the 120-kDa form is phosphorylated. After _in vivo_ labeling with [^{32}P]orthophosphate, all receptor forms were immunoprecipitated from hepatic total microsomes (TM) (with an anti-receptor antiserum), separated by NaDodSO$_4$/PAGE, and detected by autoradiography. The 120-kDa form was selectively phosphorylated, whereas the 116- and 105-kDa forms incorporated no detectable ^{32}P. To determine the topology of the phosphorylation sites, hepatic TM isolated from rats labeled _in vivo_ with either [^{35}S]cysteine or [^{32}P]orthophosphate were treated with trypsin. TM were solubilized and receptors were immunoprecipitated from lysates. With increasing trypsin concentrations, the [^{35}S]cysteine-labeled receptor triplet was degraded to a trypsin-resistant doublet of ≈95 and 85 kDa, indicating that ≈20 kDa was removed from the receptor endodomain by trypsin. The same treatment removed all detectable ^{32}P from labeled receptors. Furthermore, no ^{32}P was detected in the 80-kDa biliary form of the receptor. Serine was identified as the only phosphorylated residue in acid hydrolysates of ^{32}P-labeled immunoprecipitated receptor. These findings indicate that (*i*) the 120-kDa form is the only phosphorylated species of the receptor; and (*ii*) the phosphorylated residues are serine(s) located in the endodomain of the protein.**

Polymeric IgA [poly(IgA)], the major immunoglobulin in external secretions, is synthesized by plasma cells in the connective tissue of the mucosae of the gastrointestinal, respiratory, and urogenital tracts as well as in those of several exocrine glands (1). Once secreted from plasma cells into the local interstitial fluid, poly(IgA) is either directly transported across the cognate epithelia into external secretions or drained into the lymph and ultimately the blood (2). Circulating poly(IgA) is recovered from the blood by the liver and transported across hepatocytes to the bile, with which it eventually reaches the intestinal lumen (3). In either case, poly(IgA) receptors, synthesized by a variety of epithelial cells, including hepatocytes (4–7), mediate the transport of poly(IgA) to the external secretions apparently by receptor-mediated endocytosis (3, 8).

The biogenesis of the poly(IgA) receptor was recently studied in rat hepatocytes in our laboratory (9, 10). Our evidence, together with data obtained in systems reconstituted *in vitro* (11, 12) indicates that the poly(IgA) receptor is synthesized on polysomes attached to the endoplasmic reticulum (RER), cotranslationally inserted into the RER membrane, and glycosylated to give a 105-kDa precursor, which is transported to the Golgi complex for terminal glycosylation to generate a second, 116-kDa, precursor. Vesicular carriers transport the 116-kDa form to the sinusoidal plasmalemma (SPL), the most probable site of its slow conversion to the mature 120-kDa receptor form. It is assumed that this form binds poly(IgA), and the ensuing receptor–ligand complexes undergo internalization, apparently via coated pits and coated vesicles (8), followed by rapid transcytosis by vesicular carriers (13, 14) to the biliary plasmalemma (BPL). Upon arrival at the BPL, the receptor is proteolytically cleaved into an 80-kDa ectodomain fragment, which is secreted into the bile [either unoccupied or complexed to poly(IgA)], and a transmembrane-endodomain fragment, the fate of which is still unknown (12, 15).

The poly(IgA) receptor is unique among plasmalemmal glycoproteins in having two (instead of one) precursors. Whereas glycosylation of the receptor ectodomain is responsible for the first shift, from 105 kDa to 116 kDa (9), the nature of the second shift, from 116 kDa to 120 kDa, is still unknown, although a covalent modification such as phosphorylation may be involved. It has been shown not only that other receptors undergo phosphorylation of different residues of their endodomains as part of their functional cycle but also that phosphorylation of protein can effect a significant apparent molecular weight change (16).

Here we report that the 120-kDa form of the rat hepatic poly(IgA) receptor is preferentially phosphorylated, whereas the 116- and 105-kDa forms are not. Furthermore, the site of phosphorylation is identified as serine in the receptor endodomain.

METHODS

Materials. Reagents and supplies were obtained from the following sources: leupeptin from Boehringer Mannheim; Trasylol and phosphorylated amino acid standards from Calbiochem; phenylmethylsulfonyl fluoride from Sigma; [^{35}S]cysteine (1170 Ci/mmol; 1 Ci = 37 GBq) from Amersham; [^{32}P]orthophosphate in HCl-free solution and ^{125}I-labeled staphylococcal protein A [2–10 μCi/μg (immunological grade)] from New England Nuclear; protein A-Sepharose from Pharmacia; sheep anti-rat IgA from Cappel Laboratories (Cochranville, PA); nitrocellulose filters (BA85, 0.45 μm pores) from Schleicher & Schuell; trypsin from GIBCO; α-chymotrypsin and soybean trypsin inhibitor from Worthington; thin-layer chromatography sheets (cellulose, no fluorescent indicator) from Eastman; and NaDodSO$_4$/PAGE reagents from Bio-Rad.

Male Sprague–Dawley rats (120–150 g) and female New Zealand White rabbits (≈1.8 kg) were purchased from Camm Laboratory Animals (Wayne, NJ) and Montowese Rabbitry (Woodstock, CT), respectively.

Antibody Production. Bile was collected via a cannula (polyethylene tubing) inserted into the common bile duct in Nembutal-anesthetized rats. Bile aliquots were treated with 10 vol of ice-cold acetone to precipitate bile proteins, which

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: TM, total microsomes; RER, rough endoplasmic reticulum; SPL, sinusoidal plasmalemma; BPL, biliary plasmalemma.

were subsequently separated by $NaDodSO_4$/PAGE. An 80-kDa protein was identified as the biliary form of the poly(IgA) receptor (secretory component) by its coprecipitation with IgA, using a sheep antiserum to rat IgA. This band was excised from unstained gels and used as antigen to raise an antiserum in female New Zealand White rabbits according to a modified Papermaster *et al.* procedure (17). All antigen injections were intradermal. Bleeding was from an ear vein. The serum obtained was tested for the presence of antibodies by immunoblotting and immunoprecipitating preparations known to contain the antigen.

***In Vivo* Labeling of Animals and Preparation of Rat Liver Total Microsomes (TM).** Male Sprague–Dawley rats (120–150 g) received [^{35}S]cysteine i.v. or [^{32}P]orthophosphate i.p. (under ether anesthesia) as indicated in the figure legends. At appropriate intervals after injection, the livers were removed, placed in ice-cold buffer A (100 mM Tris·HCl, pH 7.4/250 mM sucrose supplemented with the following protease inhibitors: 0.1 mM leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1% Trasylol), and minced with scissors. The mince, washed free of blood, was homogenized in buffer A to give a 30% (wt/vol) homogenate, which was centrifuged ($7000 \times g$ for 10 min) to pellet all large particles, mitochondria included. The supernatant was centrifuged at $105,000 \times g$ for 90 min to yield a second supernatant and a TM pellet, which contains all cellular components involved in receptor synthesis and transport (smooth and rough endoplasmic reticulum, Golgi elements, vesicular carriers, and plasmalemma-derived vesicles). The TM pellet was resuspended in buffer A by gentle homogenization (18).

Immunoprecipitation. We precipitated the poly(IgA) receptor from bile proteins and TM as in ref. 9, using a 1:30 antiserum dilution to generate antigen–antibody complexes and absorbing the latter on protein A-Sepharose beads.

Immunoblotting. Immunoblotting was performed on transfers to nitrocellulose filters and quenched according to ref. 19. Antigens were detected by overlay with ^{125}I-labeled protein A followed by autoradiography.

Trypsin Treatment of Biosynthetically Labeled TM. TM were prepared from rats after injections of either [^{35}S]cysteine or [^{32}P]orthophosphate at times indicated in the figure legends and 2-mg/ml aliquots were incubated at 4°C for 60 min in buffer A minus protease inhibitors supplemented with trypsin at 0, 0.5, 5.0, 50, or 500 μg/ml in the absence or presence of 1% Triton X-100. Proteolysis was stopped by adding soybean trypsin inhibitor to a final concentration of 2.5 mg/ml, after which all TM were solubilized. Receptors were immunoprecipitated by using anti-receptor antiserum, separated by $NaDodSO_4$/PAGE, and detected by fluorography.

Gel Electrophoresis, Fluorography, and Autoradiography. $NaDodSO_4$/PAGE was carried out in 5–10% gradient gels (22 × 23 × 1.5 cm) according to ref. 20. Immunoprecipitates from TM or acetone-precipitated bile proteins were dissolved in 150 μl of electrophoresis sample buffer (0.66 M Tris·HCl, pH 6.8/2.7% $NaDodSO_4$, 67 mM dithiothreitol/8% sucrose containing bromophenol blue), boiled for 2 min, cooled, and loaded onto gels. Electrophoresis was carried out at a constant current of 25 mA until the dye front ran off of the gel. Gels were stained, destained, and subjected to fluorography as in ref. 21; autoradiographs and fluorographs of the gels were obtained on Kodak XRP film at −80°C after exposure for the times indicated in the figure legends.

The "Sandwich" Technique of Autoradiography. To detect separately ^{35}S and ^{32}P, the procedure of Axelrod (22) was used. A sandwich of the following layers was made: (*i*) the dried gel with ^{35}S- and ^{32}P-labeled samples, (*ii*) a sheet of Kodak XRP film, (*iii*) a sheet of aluminum foil to block the weak radiation from the ^{35}S label, and (*iv*) a second sheet of Kodak XRP film to detect only the strong radiation from the ^{32}P label. Autoradiographs were recorded on Kodak XRP film at −80°C after exposure for the times indicated in the figure legends.

Phosphoamino Acid Analysis. Receptors were immunoprecipitated from ^{32}P-labeled TM. Immune complexes were eluted from protein-A Sepharose beads with 1 M acetic acid (pH 1.9), lyophilized, and hydrolyzed in 200 μl of 6 M HCl for 2.5 hr at 110°C (23). The hydrolysates were lyophilized and the dried samples were dissolved in 10 μl of 2% (vol/vol) formic acid, 8% (vol/vol) acetic acid (pH 1.9). Each 10-μl sample was spotted onto one end of a cellulose plate along with 1 μl of phenol red and 1 μl each of phosphoamino acid standards (10-mg/ml stocks) and then electrophoresed at 500 V in the pH 1.9 buffer for 6 cm (≈1 hr) and in a pH 3.5 buffer [1% (vol/vol) pyridine/10% (vol/vol) acetic acid] for an additional 6 cm (≈1 hr) (H. Clive Palfrey and Angus Nairn, personal communication). Radiolabeled phosphoamino acids were located by autoradiography of the cellulose plates. Phosphoserine, phosphotyrosine, and phosphothreonine standards were located by staining with ninhydrin.

Analytical Procedures. Protein determinations were made according to ref. 24, using bovine serum albumin as a standard.

RESULTS

Phosphorylation of the Poly(IgA) Receptor *in Vivo*. The first question we asked was: Which form of the poly(IgA) receptor is phosphorylated *in vivo*? Preliminary data suggested that the 120-kDa form selectively incorporated ^{32}P, but resolution was not sufficient to eliminate the phosphorylation of the 116-kDa form. Hence, gel electrophoresis conditions were modified by using a 5–10% (rather than a 5–15%) gradient gel and by electrophoresing the dye front off of the gel. With these modifications, adequate separation of the 120- and 116-kDa bands was obtained.

To test directly whether the poly(IgA) receptor is phosphorylated *in vivo*, rats received either [^{35}S]cysteine i.v. or [^{32}P]orthophosphate i.p. according to the protocols outlined in the legend of Fig. 1. [^{35}S]Cysteine-labeled hepatic TM were prepared 30 min after a single injection, a time previously shown to be sufficient to label all three receptor forms (9). ^{32}P-labeled TM were prepared 30 min after the second injection, a time and schedule determined by preliminary

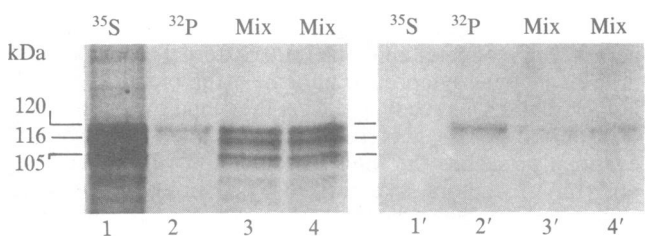

FIG. 1. Gel electrophoretic analysis of biosynthetically labeled membrane forms of the poly(IgA) receptor. Hepatic TM were prepared from rats subjected to the following injection protocols: Rat 1 received 2 mCi of [^{35}S]cysteine i.v. at time zero. At 30 min the animal was sacrificed and TM were isolated. Rat 2 received 2.5 mCi of [^{32}P]orthophosphate i.p. at time zero and again at 30 min. At 60 min the animal was sacrificed and TM were isolated. All receptor forms were immunoprecipitated from aliquots containing [^{35}S]cysteine-labeled TM alone (lane 1), ^{32}P-labeled TM alone (lane 2), and a mixture of [^{35}S]cysteine- and ^{32}P-labeled TM (lanes 3 and 4) mixed before (lane 3) or after (lane 4) immunoprecipitation. The proteins were separated by $NaDodSO_4$/PAGE and detected by fluorography and sandwich autoradiography. Exposure: 11 days at −80°C. Lanes 1–4, first x-ray film, visualizing all receptor bands labeled with ^{35}S or ^{32}P, electrophoresed separately (lanes 1 and 2) or as a mixture (lanes 3 and 4). Lanes 1′–4′, second x-ray film, visualizing receptor bands labeled with ^{32}P only.

experiments to give detectable receptor labeling. All labeled receptor forms were immunoprecipitated from solubilized TM aliquots, separated by $NaDodSO_4$/PAGE, and detected by sandwich autoradiography. Fig. 1 displays all receptor bands labeled with either [^{35}S]cysteine or $^{32}P_i$, electrophoresed separately (lanes 1, 2, 1′, and 2′) or after mixing (lanes 3, 4, 3′, and 4′). Sandwich autoradiography allowed the detection of receptor forms labeled with both ^{35}S and ^{32}P (lanes 1–4) or only ^{32}P (lanes 1′–4′). The absence of a receptor band in lane 1′ serves as a control to show that no ^{35}S radiation penetrated the aluminum foil to expose the second film. These results identify the 120-kDa receptor as a phosphoprotein on two accounts: (*i*) comigration of the ^{32}P-labeled receptor (lane 2) with the [^{35}S]cysteine-labeled 120-kDa receptor (lane 1); and (*ii*) superimposition of the ^{32}P-labeled bands (lanes 3′ and 4′) with the 120-kDa receptor band in the mixed samples (lanes 3 and 4).

To demonstrate that all receptor forms are present in ^{32}P-labeled TM, the corresponding immunoprecipitates, as well as immunoprecipitates obtained from [^{35}S]cysteine-labeled TM, were transferred to a nitrocellulose filter, which was processed through immunoblotting (with anti-receptor antiserum) followed by ^{125}I-labeled protein A and autoradiography. The results are presented in Fig. 2. The left of the figure shows the controls—i.e., direct autoradiograph of the filter, which visualizes the triplet of ^{35}S-labeled receptors (lane 1) and the ^{32}P-labeled 120-kDa form (lane 2). The right shows the same filter after immunoblotting (and processing as above). Although the 120-kDa and 116-kDa bands are not clearly resolved, all three receptor forms were detected in [^{35}S]cysteine-labeled (lane 1′) and ^{32}P-labeled (lane 2′) TM.

Topology of the Phosphorylation Sites. The next question we asked was: Which domain of the receptor is phosphorylated? The inquiry is relevant, since the poly(IgA) receptor is a transmembrane protein (9, 11, 12), and since phosphorylation, presumed to be connected with the regulation of their function, is thought to affect the endodomains of other transmembrane receptors (25). The experiments used trypsin treatment of intact TM biosynthetically labeled with either [^{35}S]cysteine or [^{32}P]orthophosphate, followed by solubilization and immunoprecipitation of receptors and ectodomain fragments, which were separated by $NaDodSO_4$/PAGE and detected by fluorography (^{35}S) and autoradiography (^{32}P). The results are shown in Fig. 3. With increasing concentrations of trypsin (lanes 1–4), the ^{35}S-labeled receptor triplet, 120, 116, and 105 kDa, was reduced to a doublet of ≈95 and

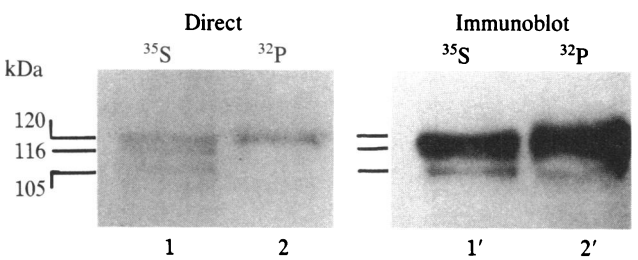

FIG. 2. Gel electrophoretic and immunoblotting analysis of biosynthetically labeled membrane forms of the poly(IgA) receptor. Hepatic TM were prepared from rats subjected to the injection protocols outlined in the legend of Fig. 1. Receptors were immunoprecipitated from either [^{35}S]cysteine- or ^{32}P-labeled TM, and the immunoprecipitates were separated by $NaDodSO_4$/PAGE and transferred to a nitrocellulose filter. The transfer was subjected to autoradiography. Exposure: 5 days at −80°C to detect biosynthetically labeled receptor forms (lanes 1 and 2). To check for the presence of all receptor forms regardless of their incorporated biosynthetic labels, the same transfer was subsequently subjected to immunoblotting with anti-receptor antiserum followed by overlay with ^{125}I-labeled protein A and autoradiography (lanes 1′ and 2′). Exposure: 17 hr at −80°C.

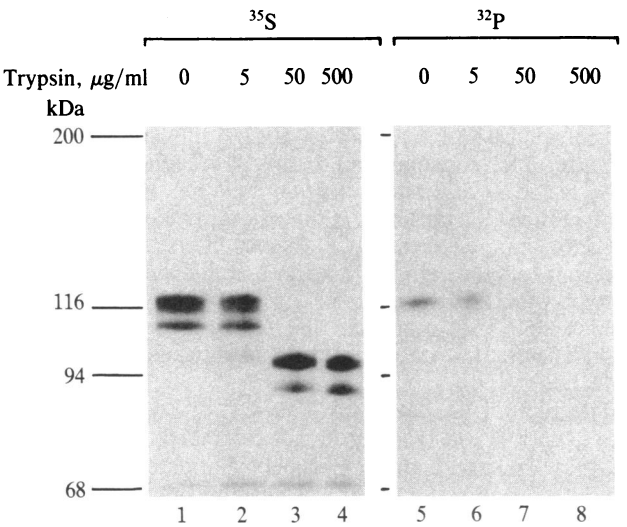

FIG. 3. Gel electrophoretic analysis of biosynthetically labeled poly(IgA) receptors before and after trypsin digestion of TM. Hepatic TM were prepared from rats subjected to the injection protocols outlined in the legend of Fig. 1. TM aliquots (2 mg/ml) were incubated at 4°C for 60 min in buffer A minus protease inhibitors and supplemented with trypsin at the indicated concentrations. Proteolysis was stopped by adding soybean trypsin inhibitor to a final concentration of 2.5 mg/ml. All receptor forms were immunoprecipitated from solubilized TM, separated by $NaDodSO_4$/PAGE, and detected by fluorography (lanes 1–4) or autoradiography (lanes 5–8). Exposure: 3 days at −80°C in the case of ^{35}S-labeled receptors and for 14 days at −80°C for ^{32}P-labeled receptors. Lanes 1 and 5, immunoprecipitates from untreated microsomes; lanes 2–4 and 6–8, immunoprecipitates from microsomes treated with increasing concentrations of trypsin.

≈85 kDa (lanes 3 and 4). Consistent with previously published results (9, 12), the loss of ≈20 kDa from the receptor triplet presumably is due to the removal of the receptor endodomain. The resistance of this doublet to further proteolysis was dependent on microsomal vesicle integrity, since solubilization of the microsomes with Triton X-100 prior to the addition of trypsin generated smaller fragments (data not shown). Identical trypsin treatment of ^{32}P-labeled TM resulted in a loss of ^{32}P label from the receptor, as shown in lanes 5–8.

The interpretation of a the proteolysis data takes into account the fact that our antibody recognizes only the ectodomain of the poly(IgA) receptor and not the endodomain. Furthermore, a TM preparation is a mixed vesicular fraction in which receptors assume two transmembrane orientations. First, in microsomal vesicles derived from intracellular compartments (i.e., RER, Golgi complex, and vesicular carriers), receptors are expected to have their ectodomains (ligand binding domains) facing the vesicle lumen and their endodomains (cytoplasmic tails) facing out. Second, receptors in plasmalemma-derived microsomal vesicles are expected to have a converse orientation: ectodomain out and endodomains facing the lumen. Trypsin digestion of the vesicles derived from intracellular compartments should cleave off the exposed receptor endodomain, leaving the ectodomains (and transmembrane domains) protected by the vesicular membrane. After subsequent solubilization of these trypsin-treated TM, only ectodomain fragments should be available for immunoprecipitation. Our data (Fig. 3) demonstrate that the loss of ^{32}P label from the receptor (lanes 5–8) occurs under conditions in which the receptor endodomain is cleaved off (lanes 1–4), suggesting that the phosphorylated residues are on the endodomain. Presumably, the ^{32}P label is lost primarily from receptors residing in transcellular vesicular carriers involved in transferring receptors and receptor-

ligand complexes from the SPL, the putative cellular site of receptor phosphorylation, to the BPL and not from other intracellular compartments or carriers.

A TM fraction also contains a small component of plasmalemma-derived vesicles [≈10% of TM membrane (26)], in which phosphorylated 120-kDa receptors are expected to reside in an ectodomain-out orientation. However, after trypsin digestion, residual ^{32}P label ascribable to such vesicles was not detected. We assume either that antigenic determinants on exposed receptor ectodomains were affected by proteolysis and no longer precipitable by our antibody (which does not recognize membrane-protected endodomains) or that the amount of residual antigens is too low for detection.

The proteolysis data were checked by experiments designed to find out whether or not the receptor ectodomain (80-kDa biliary receptor form) is phosphorylated *in vivo*. Rats received injections of either [^{35}S]cysteine or [^{32}P]orthophosphate as outlined in the legend of Fig. 4. This injection protocol ensured that transmembrane receptors (120, 116, and 105 kDa) and the biliary receptor (80 kDa) would be biosynthetically labeled with [^{35}S]cysteine, as determined by previous kinetic experiments (9, 10). Receptors were immunoprecipitated from [^{35}S]cysteine and ^{32}P-labeled TM and bile (B), separated by $NaDodSO_4$/PAGE, and detected either by fluorography or by immunoblotting. The results are presented in Fig. 4. The left six lanes are two fluorographs showing that (*i*) [^{35}S]cysteine was incorporated into the transmembrane triplet (lane 1) and the biliary form of the receptor (lane 2); (*ii*) the 120-kDa form selectively incorporated ^{32}P (lane 3); and (*iii*) ^{32}P did not detectably label the 80-kDa biliary receptor (lane 4). To demonstrate the presence of the biliary receptor in ^{32}P-labeled bile, immunoprecipitates obtained from ^{32}P- and ^{35}S-labeled bile proteins were transferred to a nitrocellulose filter and subjected to immunoblotting with anti-receptor antiserum followed by ^{125}I-labeled protein A overlay and autoradiography. The right of Fig. 4 is an autoradiograph confirming the presence of the biliary receptor (lanes 6 and 8) as well as the receptor triplet (lanes 5 and 7) in immunoprecipitates prepared from biosynthetically labeled TM and bile. Thus, the lack of ^{32}P label in the biliary receptor supports the conclusion of the proteolysis data (Fig. 3) that the phosphorylated residues are on the receptor endodomain.

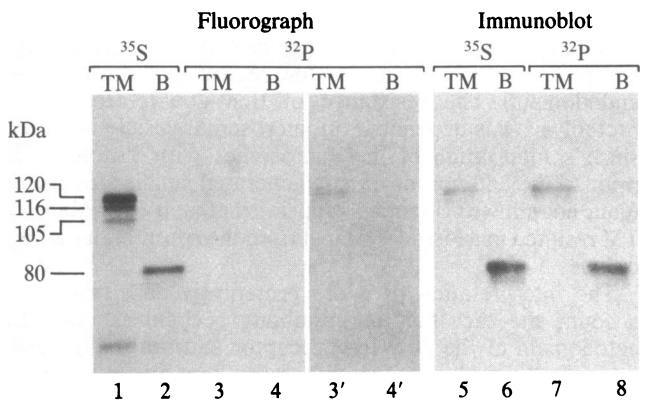

FIG. 4. Gel electrophoretic and immunoblotting analysis of biosynthetically labeled membrane and biliary forms of the poly(IgA) receptor. Rats were subjected to the following protocol: Their bile ducts were cannulated and bile was collected throughout the experiment in aliquots of 200 μl/10 min. Thirty minutes later (time zero), rat 1 received 2.5 mCi of [^{35}S]cysteine i.v.; the injection was repeated at 40 min. Rat 2 received 2.5 mCi of [^{32}P]orthophosphate i.p. at time zero and again 40 min later. At 80 min the rats were sacrificed and their hepatic TM were isolated. All receptor forms were immunoprecipitated from [^{35}S]cysteine- and ^{32}P-labeled TM and from acetone-precipitated bile proteins. All bile aliquots gave the same result. Only the 60- to 70-min aliquot is shown. One set of immunoprecipitates was separated by $NaDodSO_4$/PAGE and detected by fluorography. Exposure: 12 days (lanes 1–4) or 4 months (lanes 3′ and 4′) at −80°C. A duplicate set of immunoprecipitates was processed through $NaDodSO_4$/PAGE, transfer to a nitrocellulose filter, immunoblotting of the transfers with anti-receptor antiserum followed by ^{125}I-labeled protein A overlay, and autoradiography. Exposure: 5 hr at −80°C (lanes 5–8). Lanes 1, 3, 3′, 5, and 7 contain transmembrane receptor forms immunoprecipitated from TM. Lanes 2, 4, 4′, 6, and 8 contain the secreted biliary receptor immunoprecipitated from bile (B).

Phosphoamino Acid Analysis. The next question to be answered pertained to the identification of the phosphorylated amino acid(s) of the receptor. To this intent, immunoprecipitates of ^{32}P-labeled receptors were subjected to acid hydrolysis followed by one-dimensional thin-layer electrophoresis. The autoradiograph in Fig. 5 (lane 1) shows that phosphoserine is the only radioactive phosphoamino acid present as determined by comigration of the ^{32}P label with the phosphoserine standard (lane 2).

DISCUSSION

Three lines of investigation suggest that the rat hepatic poly(IgA) receptor is phosphorylated on serine residue(s) in the endodomain: (*i*) the mature 120-kDa transmembrane receptor is labeled *in vivo* with [^{32}P]orthophosphate (Figs. 1 and 2) but the 80-kDa biliary receptor (secreted ectodomain, presumably of the 120-kDa form) is not (Fig. 4); (*ii*) trypsin digestion of biosynthetically labeled TM removes an ≈20-kDa endodomain and concomitantly there is a complete loss of ^{32}P label from the receptor (Fig. 3); and (*iii*) phosphoamino acid analysis of immunoprecipitated receptors has identified serine as the phosphorylated residue (Fig. 5).

These findings are of general interest since the phosphorylation of different amino acid residues of the endodomain is a common trait for many plasmalemmal receptors. In

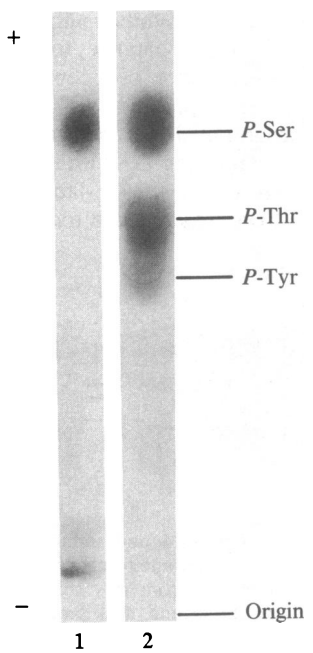

FIG. 5. Autoradiograph of ^{32}P-labeled phosphoamino acids separated by one-dimensional thin-layer electrophoresis. Receptors were immunoprecipitated from ^{32}P-labeled TM. Immune complexes were eluted from protein A-Sepharose beads with 1 M acetic acid, lyophilized, and subjected to acid hydrolysis. Hydrolysates were analyzed by one-dimensional thin-layer electrophoresis (lane 1). The phosphoamino acid standards were located by ninhydrin staining (lane 2). Exposure: 14 days at −80°C.

addition to the rat hepatic poly(IgA) receptor, the receptors of rat asialoglycoprotein (27), human asialoglycoprotein (28), human transferrin (29), and rat IgE (30) are phosphorylated on serine residues, as are the chicken hepatic lectin (25) and the hamster β-adrenergic receptor (31). The receptors for epidermal growth factor (32) and insulin (33, 34) also are phosphorylated on serine residues in the absence of ligand, but in the presence of ligand, they undergo autophosphorylation of tyrosine residues (34, 35). Similarly, platelet-derived growth factor (PDGF) stimulates the phosphorylation of tyrosine residues in several membrane proteins in a receptor-dependent manner. Whether PDGF stimulates autophosphorylation of its receptor is still unclear (36, 37).

The biological function of phosphorylation of the poly(IgA) receptor has yet to be defined. For other receptors, the literature postulates that phosphorylation, especially affecting tyrosine residues, plays a role in signal transduction, which in some cases initiates a cascade of phosphorylation–dephosphorylation reactions and a multiplicity of cellular responses. The latter may include general phenomena such as receptor clustering into coated pits and internalization via coated vesicles. The assumption is plausible, since tyrosine-phosphorylated receptors are involved in receptor-mediated endocytosis. Alternatively, phosphorylation of specific endodomain residues may be involved in protein sorting or control of vesicular traffic within the cells. A general, rather than specific, role is suggested by the fact that, at the present level of inquiry, phosphorylation of similar residues coexists with different types of sorting. For example, receptors for asialoglycoprotein (28) and poly(IgA) (current study) are phosphorylated on serine residues, but their sorting patterns differ. Asialoglycoprotein receptors constitutively recycle from the cell surface to an acidic compartment (where ligand–receptor uncoupling occurs if the receptor is occupied) and back to the cell surface. In contrast, the poly(IgA) receptor is not recycled, but it is transferred, either without ligand or complexed to ligand, from the SPL to the BPL prior to proteolytic cleavage and secretion. It is possible, however, that such differences are accounted for by the phosphorylation of specific serine residues within the corresponding endodomains. Further studies are necessary to elucidate where in the cell, under what conditions, and with what consequences phosphorylation of the poly(IgA) receptor occurs.

Note Added in Proof. In recent experiments, we found that alkaline phosphatase treatment of immunoprecipitated receptors causes (*i*) a loss of ^{32}P label from the 120-kDa form and (*ii*) the collapse of the [^{35}S]cysteine-labeled triplet to a 116/105-kDa doublet. These data support the hypothesis that phosphorylation is responsible for the shift from 116 kDa to 120 kDa.

We thank Dr. Richard Huganir of The Rockefeller University for his invaluable help with the phosphoamino acid analysis. We acknowledge Linda S. Ketchman for excellent technical assistance, Cindy Davis for secretarial assistance, and Pam Ossorio for photography. This research was supported by a grant from the National Institutes of Health (GM 27303). J.M.L. is the recipient of a National Research Service Award Postdoctoral Fellowship from the National Institute of General Medical Sciences (1 F 32 GM 10723-01).

1. Hanson, L. A., Ahlstedt, S., Andersson, B., Carlsson, B., Cole, M. F., Cruz, J. R., Dahlgren, V., Ericsson, T. H. & Jalil, F. (1983) *Ann. N. Y. Acad. Sci.* **409**, 1–21.
2. Kleinman, R. E., Harmatz, P. R. & Walker, W. A. (1982) *Hepatology* **2**, 379–384.
3. Orlans, E., Peppard, J. V., Payne, A. W. R., Fitzharris, B. M., Mullock, B. M., Hinton, R. H. & Hall, J. G. (1983) *Ann. N. Y. Acad. Sci.* **409**, 411–427.
4. Tomasi, T. B., Tan, E. M., Soloman, A. & Prendergast, R. A. (1965) *J. Exp. Med.* **212**, 101–124.
5. Fisher, M. M., Nagy, B., Bazin, H. & Underdown, B. J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2008–2012.
6. Orlans, E., Peppard, J., Reynolds, J. & Hall, J. (1978) *J. Exp. Med.* **147**, 588–592.
7. Lemaitre-Coelho, I., Altamirano, G. A., Barranco-Acosta, C., Meykens, R. & Vaerman, J.-P. (1981) *Immunology* **43**, 261–270.
8. Courtoy, P. J., Limet, J. N., Quintart, J., Schneider, Y.-J., Vaerman, J.-P. & Baudhuiin, P. (1983) *Ann. N. Y. Acad. Sci.* **409**, 799–802.
9. Sztul, E. S., Howell, K. E. & Palade, G. E. (1984) *J. Cell Biol.* **100**, 1248–1254.
10. Sztul, E. S., Howell, K. E. & Palade, G. E. (1984) *J. Cell Biol.* **100**, 1255–1261.
11. Mostov, K. E., Kraehenbuhl, J.-P. & Blobel, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7257–7261.
12. Mostov, K. E. & Blobel, G. (1982) *J. Biol. Chem.* **257**, 11816–11821.
13. Takahashi, I., Nakane, P. K. & Brown, W. R. (1982) *J. Immunol.* **128**, 1181–1187.
14. Renston, R. H., Jones, A. L., Christiansen, W. D. & Hradek, G. T. (1980) *Science* **208**, 1276–1278.
15. Delacroix, D. L., Hodgson, H. J. F., McPherson, A., Dive, C. & Vaerman, J.-P. (1982) *J. Clin. Invest.* **70**, 230–241.
16. Wang, S.-Y. & Williams, D. L. (1982) *J. Biol. Chem.* **257**, 3837–3846.
17. Papermaster, D. S., Converse, C. A. & Zorn, M. A. (1976) *Exp. Eye Res.* **23**, 105–116.
18. Ehrenreich, J. H., Bergeron, J. J. M., Siekevitz, P. & Palade, G. E. (1973) *J. Cell Biol.* **59**, 45–72.
19. Gershoni, J. M. & Palade, G. E. (1982) *Anal. Biochem.* **124**, 396–405.
20. Maisel, J. V. (1971) in *Methods in Virology*, eds. Maramorosh, K. & Kiprowski, K. (Academic, New York), Vol. 5, pp. 179–246.
21. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
22. Axelrod, N. (1978) *Virology* **87**, 366–383.
23. Huttner, W. B., DeGennaro, L. J. & Greengard, P. (1981) *J. Biol. Chem.* **255**, 1482–1488.
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
25. Drickamer, K. & Mamon, J. F. (1982) *J. Biol. Chem.* **257**, 15156–15161.
26. Wibo, M., Amar-Costesec, A., Berthet, J. & Beaufay, H. (1971) *J. Cell Biol.* **51**, 52–71.
27. Takahashi, T., Nakada, H., Okumura, T., Sawamura, T. & Tashiro, Y. (1985) *Biochem. Biophys. Res. Commun.* **126**, 1054–1060.
28. Schwartz, A. L. (1984) *Biochem. J.* **223**, 481–486.
29. Schneider, C., Sutherland, R., Newman, R. & Greaves, M. (1982) *J. Biol. Chem.* **257**, 8516–8522.
30. Fewtrell, C., Goetze, A. & Metzger, H. (1982) *Biochemistry* **21**, 2004–2010.
31. Benovic, J. L., Pike, L. J., Cerione, R. A., Staniszewski, C., Yoshimasa, T., Codina, J., Caron, M. G. & Lefkowitz, R. L. (1985) *J. Biol. Chem.* **260**, 7094–7101.
32. Carlin, C. R., Phillips, P. D., Knowles, B. B. & Cristofalo, V. J. (1983) *Nature (London)* **306**, 617–620.
33. Kasuga, M., Zick, Y., Blithe, D. L., Karlsson, F. A., Haring, H.-U. & Kahn, C. R. (1982) *J. Biol. Chem.* **257**, 9891–9894.
34. Pang, D. T., Sharma, B. R., Shafer, J. A., White, M. F. & Kahn, C. R. (1985) *J. Biol. Chem.* **260**, 7131–7136.
35. Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255**, 8363–8365.
36. Pike, L. J., Bowen-Pope, D. F., Ross, R. & Krebs, E. G. (1983) *J. Biol. Chem.* **258**, 9383–9390.
37. Ek, B., Westermark, B., Wateson, A. & Heidin, C.-H. (1982) *Nature (London)* **295**, 419–420.