Membrane and soluble forms of alkaline phosphatase (ALP) were selectively prepared from human placental microsomes by treatment with 1-butanol at pH 8.5 and 5.5, respectively. The purified membrane (mALP) and soluble (sALP) forms were analyzed for chemical compositions. mALP was found to contain 1 mol each of palmitate, stearate, and glycerol/subunit of ALP, which were absent in sALP. Both the forms contained 1 mol of inositol and 2 mol of ethanolamine/subunit. However, none of these compounds was detectable in another soluble form prepared by treatment with papain, which is known to cleave the carboxyl-terminal region. The results suggest that mALP contains diacylglycerol, the removal of which results in its conversion to sALP. We then prepared [3H]ethanolamine-labeled ALP by incubating choriocarcinoma cells (JEG-3) with the isotope. [3H]-Labeled sALP was mixed with unlabeled sALP and treated with papain. A [3H]-labeled single component was purified from the digests by sequential chromatography through anti-ALP-IgG-Sepharose, concanavalin A-Sepharose, Bio-Gel P-6, and TSK G-2000 columns. Chemical analyses revealed that the purified sample contains the tripeptide Thr-Thr-Asp, ethanolamine, glucosamine, mannose, inositol, and phosphate. Molar ratios of the latter five components were calculated to be 2, 1, 3, 1, and 2, respectively, by taking Asp as 1 mol. The tripeptide sequence was identified at positions 482–484 in the primary structure deduced from the cDNA sequence, which predicts a further extension to position 513, containing a hydrophobic amino acid sequence. Taken together, these results suggest that the mature ALP molecule lacks the predicted carboxyl-terminal peptide extension and is attached at Asp with a glycosylphospholipid, the components of which are characterized above. The glycosylphospholipid thus attached is considered to function as the membrane anchor of ALP. of at least three tissue-specific forms or isozymes: tissue-unspecific (found in liver, kidney, bone, etc.), intestinal, and placental types (1–3). These isozymes are well characterized by enzymological and immunochemical approaches (1). Most recently, cDNAs for tissue-unspecific (4), intestinal (5), and placental (6–8) isozymes have been isolated. The sequence analyses of these cDNAs predicted their structural details, revealing the difference and homology in primary structure among the three isozymes.

ALP is a membrane-bound glycoprotein and a marker enzyme for the plasma membrane (1). In fact, all the primary structures of these isozymes deduced from their cDNAs are found to contain at the carboxyl terminus a hydrophobic domain which could participate in membrane localization (4–8), as observed for other membrane proteins (9). On the other hand, it is now established that ALP is released from the membrane by phosphatidylinositol-specific phospholipase C. This was initially demonstrated by Slein and Logan (10), who used a partially purified phospholipase, and subsequently confirmed in a wide range of animal tissues by use of highly purified phosphatidylinositol-specific phospholipase C (11–13). Thus, attachment of ALP (at least the mature form) to the membrane is suggested to involve phosphatidylinositol instead of its hydrophobic peptide domain at the carboxyl terminus predicted from the cDNA. Involvement of phosphatidylinositol is also suggested for other membrane-bound proteins (14, 15). Recently, hydrophobic carboxyl-terminal domains were isolated from the variant surface glycoprotein (VSG) of Trypanosoma brucei (16), Thy-1 (17), acetylcholinesterase (18), and decay-accelerating factor of complement (19). Chemical analyses revealed that they contain a novel glycosylphospholipid, including ethanolamine, neutral sugars, hexosamine, and phosphatidylinositol, which is proposed as the membrane anchor. However, no detailed chemical data have been provided for the membrane-anchoring domain of ALP.

In this study, we prepared membrane and soluble forms of human placental ALP and further isolated the carboxyl-terminal fragment, demonstrating details of their chemical compositions.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fresh human term placentas were obtained from the Fukuoka University Hospital. All the placentas used in this study were obtained within 2 h after delivery. The human choriocarcinoma cell line JEG-3 was supplied by Dr. S. Sekiya (Department of Obstetrics and Gynecology, Chiba University School of Medicine). Phosphatidylinositol-specific phospholipase C was purified from Bacillus cereus as described previously (13). Papain and papain-agarose were obtained from Sigma; [3H]ethanolamine (12.0 Ci/mmol) was from Amerham Corp.; concanavalin A (ConA)-Sepharose and Sephacryl S-300 were from Bio-Rad; and antiphosphatase was from Bio-Rad. Antiplacental ALP-IgG and an immu-

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Human alkaline phosphatase (ALP)† (EC 3.1.3.1) consists of at least three tissue-specific forms or isozymes: tissue-unspecific (found in liver, kidney, bone, etc.), intestinal, and placental types (1–3). These isozymes are well characterized by enzymological and immunochemical approaches (1). Most recently, cDNAs for tissue-unspecific (4), intestinal (5), and placental (6–8) isozymes have been isolated. The sequence analyses of these cDNAs predicted their structural details, revealing the difference and homology in primary structure among the three isozymes.

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In this study, we prepared membrane and soluble forms of human placental ALP and further isolated the carboxyl-terminal fragment, demonstrating details of their chemical compositions.
Preparation of Membrane and Soluble Forms of ALP—Crude microsomes were prepared from human placenta and subjected to selective preparation of membrane and soluble forms of ALP as described previously (20, 21). In brief, for preparation of mALP, the membrane suspension (2-3 mg/ml) was adjusted to pH 5.5 with 1 M acetic acid (final concentration of 50 mM) and treated with 1-butanol (25%) at 25 °C for 1 h. sALP was extracted with 1-butanol as described above after the membrane suspension had been adjusted to pH 5.5 with 1 M acetic acid (final concentration of 50 mM) and extracted with 1-butanol (25%) at 25 °C for 1 h.

Purification of Soluble and Membrane Forms of ALP—Two forms (sALP and mALP) of placental ALP were purified by essentially the same method as that used for purification of rat ALP (21). In brief, sALP extracted from the membranes with 1-butanol at pH 5.5 was purified by sequential chromatography through ConA-Sepharose S-300, and hydroxylapatite columns. sALP thus purified was used for production of anti-ALP antibodies in rabbits, followed by preparation of an immunoaffinity column (21, 22). Once the immunoaffinity column was prepared, it was used for purification of mALP. ConA-Sepharose chromatography through ConA-Sepharose S-300 was used for production of anti-ALP antibodies in rab. The same method used for sALP mALP obtained by 1-butanol extraction at pH 8.5 was also purified by successive chromatography through ConA-Sepharose and immunoaffinity columns in the presence of 0.1% Triton X-100.

Preparation of [3H]Ethanolamine-labeled ALP—Since the available evidence (14, 15) suggests that the carboxyl terminus of placental ALP is covalently attached to ethanolamine, we prepared [3H]ethanolamine-labeled ALP. JEG-3 chorionic carcinoma cells (5 × 10^6 cells/60 mm dish) were incubated at 37 °C for 14 h with [3H]ethanolamine (500 μCi/dish) (24). The cells were separated, washed, and mixed with the unlabeled placental microsomes (20 mg) in 2 ml of distilled water. The mixture was adjusted to pH 5.5 with 1 M acetate buffer (final concentration of 50 mM) and treated with 1-butanol (25%), followed by purification of sALP as described above.

Isolation of Carboxyl-terminal Fragment—The radioactivity of [3H]ethanolamine incorporated into ALP was monitored in the following steps for purification of its carboxyl-terminal fragment. [3H]-Labeled sALP was mixed with purified unlabeled sALP (8–10 μg/ml), and the mixture was incubated at 25 °C for 5 h with papain-agarose (10 μg/ml). After removal of papain-agarose by centrifugation, the supernatant was applied to an anti-ALP IgG-Sepharose column (1.5 × 10 cm), which was washed with 20 mM Tris-Cl (pH 7.5), 0.5 mM NaCl, 1 mM MgCl₂. The radioactive flow-through fraction was collected and applied to a ConA-Sepharose column (1.1 × 6.0 cm) previously equilibrated with 20 mM Tris-Cl (pH 7.5), 0.5 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂. The column was washed with 80 ml of the above solution, followed by elution with 0.3 M methyl-α-D-glucoside in 20 mM Tris-Cl (pH 7.5), 0.5 mM NaCl. A radioactive peak eluted with the competitor was pooled and concentrated to dryness by a SpeedVac concentrator (Savant Instruments, Inc., Hicksville, NY). The dried sample was dissolved in 2 ml of 0.1 M NH₄HCO₃, applied onto an Bio-Gel P-6 column (1.6 × 80 cm), and eluted with the same solution. A major radioactive peak was obtained at a position close to the elution position of desialylated glycopeptide of rat C3 (26). Fractions in the radioactive peak were pooled and concentrated to dryness. The dried samples were dissolved in 0.2 ml of 0.1 M CH₃COOH and subjected to high performance liquid chromatography (HPLC) through a TSK G-2000SW column (0.75 × 60 cm; Toyo Soda Kogyo, Tokyo, Japan) in 0.1 M CH₃COOH. The elution profile was monitored by a UV detector at 254 nm and fractions of 0.55 ml were collected, followed by determination of radioactivity. A single radioactive peak was obtained at fractions 32–35, which were pooled and concentrated to dryness. The samples thus purified were dissolved in 2 ml of distilled water and used for chemical analyses.

Amino Acid Composition and Sequence—Primary amino acids, glucosamine, and ethanolamine were quantitated by a Hitachi amino acid analyzer (Model 835) as described previously (22). Samples were hydrolyzed in 6 M HCl at 110 °C in vacuo for 24 h. For correction of glucosamine values, samples were hydrolyzed in 3 M p-toluensulphonic acid at 110 °C for 24 h (26). Purified samples of ALP and ALP-derived peptides were also analyzed for their sequences by an Applied Biosystems Model 477A gas-phase sequencer with an on-line Model 120A phenylthiohydantoin derivative analyzer using the manufacturer's protocol (27).

Analysis of Fatty Acids and Glycerol—Noncovalently attached lipids were completely removed from the purified mALP, sALP, or psALP as described previously (28, 29). For analysis of fatty acids, samples (6 mg each) were hydrolyzed under N₂ in 1 ml of 6 M HCl for 5 h at 100 °C (28). The hydrolysates were extracted with hexane, and the extracted samples were treated with 12% BF₃ in methanol for 5 min at 100 °C to obtain fatty acid methyl esters (28). The samples were analyzed by gas-liquid chromatography on a Shimadzu GC-7A apparatus as described previously (29). Methyl esters were identified by their retention times in comparison with those of authentic methyl fatty acids (myristic acid, palmitic acid, and stearic acid) and quantitated by using heptadecanoic acid as the internal standard. Samples for glycerol analysis were hydrolyzed in 6 M HCl at 100 °C for 24 h. Glycerol was quantitated by an enzymatic assay (30) using a Hitachi 228 spectrophotometer to monitor continuously the change in absorbance at 540 nm due to the oxidation of NADH. A known amount of glycerol was added to sample cuvettes after the experimental glycerol determination to check that no enzyme inhibitors were present in the samples used.

Neutral Sugar Analysis—For analysis of inositol, samples were hydrolyzed under N₂ in 1 ml of 6 M HCl at 100 °C for 24 h (17) with L-arabinose as internal standard. Neutral sugars were separated from amino sugars as described previously (22). Inositol was identified and quantitated by gas-liquid chromatography after monosaccharides were reduced and trifluoroacetylated (22, 29). For determination of other neutral sugars, samples were hydrolyzed in 2.5 M trifluoroacetic acid at 100 °C for 8 h (30). Neutral sugars were separated from amino sugars and analyzed by the same procedure as described above.

Other Assay Methods—Phosphatase in samples which had been hydrolyzed in 6 M HCl at 100 °C for 24 h was quantitated by the Triton X-100 turbidity method involving formation of a phosphate-molybdate complex (31). The ALP activity was determined with p-nitrophenyl phosphate as substrate (32). Protein was determined by the method of Lowry et al. (33) with bovine serum albumin as standard.

Polyacrylamide Gel Electrophoresis—Standard polyacrylamide gel (7.5%) electrophoresis was carried out at pH 8.5 in the presence or absence of 0.1% Triton X-100 (32). SDS-polyacrylamide gel (9.0%) electrophoresis was performed according to the method of Laemmli (34). Gels were stained for protein with Coomassie Brilliant Blue. Apparent molecular weights were determined by coelectrophoresis of the marker proteins myosin (200,000), β-galactosidase (116,000), phosphorylase b (92,500), bovine serum albumin (66,000), and ovalbumin (45,000).

RESULTS

Release of ALP from Placental Microsomes—We previously established the method for selective preparation of membrane and soluble forms of ALP from rat tissues (20, 21). The same method was found to be applicable for human placental ALP; mALP and sALP were prepared by treatment of microsomes with 1-butanol at pH 5.5 and 5.5, respectively. Placental ALP was also effectively released as a soluble form from membrane preparations by treatment with phosphatidylinositol-specific phospholipase C or papain. Four preparations of ALP obtained by these treatments were compared by polyacrylamide gel electrophoresis in the absence of detergents (Fig. 1). mALP (lane 1) did not migrate into gels, possibly due to its aggregation; whereas sALP as well as two other enzymatically released ALPs (lanes 2–4) migrated as a single band with the same mobility.

Purification of Placental ALP—sALP was relatively easily purified from microsomes by a conventional method. Once the immunoaffinity column was available, mALP as well as sALP could be purified with much better yields without the use of chromatography through Sephacryl S-300 and hydroxylapatite columns. Approximately 15 mg of the purified ALP in

Membrane-Anchoring Domain of Alkaline Phosphatase
either form was obtained from a single placenta, and each form had a similar specific activity of 900 units (μmol/min)/mg of protein. We finally prepared about 500 mg each of mALP and sALP by repeating the purification steps. Two other soluble forms obtained by treatment with phosphatidylinositol-specific phospholipase C or papain were also purified. Polyacrylamide gel electrophoresis (Fig. 2) demonstrated a single component in each purified sample. In the presence of Triton X-100 (Fig. 2A), mALP (lane 1) migrated more slowly than the three other soluble forms (lanes 2–4). Apparent molecular weight of these forms were determined by SDS-polyacrylamide gel electrophoresis (Fig. 2B): mALP (67,000), sALP (68,000), phosphatidylinositol-specific phospholipase C-treated ALP (68,000), and psALP (66,000). Thus, all four forms have similar molecular weights, in contrast to the great difference between mALP and the other soluble forms in electrophoresis in the absence (Fig. 1) or presence (Fig. 2A) of Triton X-100. It is likely that such a characteristic behavior of mALP is due to its hydrophobic nature.

**Amino Acid Composition and Amino-terminal Sequence**—
The three purified forms (mALP, sALP, and psALP) were analyzed for amino acid composition (Table I). It was found that all three forms contain almost the same amino acid composition. Values of each residue are reasonably consistent with those in the primary structure (residues 1–484) predicted by the cDNA sequence (6–8). Furthermore, amino-terminal sequences determined up to the 30th residue in each form showed the same sequence starting with Ile (data not shown).

The results indicate that the three forms have almost identical polypeptide chains with the same amino terminus.

**Identification of Characteristic Components for Glycosylphospholipid Anchor**—mALP has been suggested to attach to phosphatidylinositol in the membrane (20–13). The three purified forms (mALP, sALP, and psALP) were analyzed for possible components of glycosylphosphatidylinositol. The results obtained are summarized in Table II. The difference between mALP and sALP is characterized by the presence of
TABLE II
Comparison of chemical compositions among three forms of placental ALP

<table>
<thead>
<tr>
<th>Component</th>
<th>mALP</th>
<th>sALP</th>
<th>psALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>0.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>0.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Inositol</td>
<td>0.9</td>
<td>0.8</td>
<td>ND</td>
</tr>
<tr>
<td>Phosphate</td>
<td>3.8</td>
<td>3.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>2.2</td>
<td>2.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detectable.

1 mol each of palmitate, stearate, and glycerol/subunit in mALP, although both forms contain equal amounts of inositol (1 mol) and ethanolamine (2 mol). In addition, mALP contains about 4 mol of phosphate, whereas sALP contains about 3 mol. In contrast, psALP contains none of these components except for 1 mol of phosphate. These results suggest that mALP has a dicarboxylic moiety characteristic for a membrane form, the removal of which may result in its conversion to sALP. The absence of these components in psALP suggests that they attach to the carboxyl terminus of ALP because papain is known to cleave its carboxyl terminus (24, 35).

The presence of phosphate (1 mol) in psALP indicates the possibility that the polypeptide chain itself is phosphorylated.

Isolation of [3H]Ethanolamine-labeled Carboxy-terminal Fragment—Since the chemical data indicate that sALP contains the characteristic component ethanolamine possibly at its carboxyl terminus, we prepared [3H]ethanolamine-labeled sALP, from which a 3H-labeled fragment was isolated after treatment with papain. About 80% of the radioactivity flowed through the immunoaffinity column, onto which the papain-digested sample was applied. When the flow-through fraction was subjected to the ConA-Sepharose column, about a half of the total radioactivity applied flowed through the column, and the remaining activity was eluted from the column with 0.3 M methyl-a-D-glucoside. Subsequent Bio-Gel P-6 chromatography showed that the radioactivity of the latter was detected at an elution position close to that of rat C3 glycoprotein with M, 1800 (25), whereas the radioactivity of the flow-through fraction was at a position with M, <1000. Since the flow-through fraction was further confirmed not to contain a significant amount of amino acids, its radioactivity may be mostly due to free [3H]ethanolamine which had not been completely removed from the 3H-labeled sALP during these isolation steps.

The sample obtained by Bio-Gel P-6 chromatography was then subjected to HPLC through a TSK G-2000 column. As shown in Fig. 3, it was separated into two major and several minor peaks when monitored by absorbance at 225 nm, and a single radioactivity peak was obtained at the second major peak of the absorbance profile. Fractions of the radioactivity peak were pooled, desalted, and subjected to HPLC through a microBondapak C18 column, resulting in a single peak with radioactivity and absorbance at 225 nm without retention (data not shown). Thus, the radioactive fraction obtained by HPLC with a TSK G-2000 column was considered to contain a single component. These purification steps starting with 8-10 mg of sALP yielded at least 50 nmol of the purified component on the basis of the Asp content (see Fig. 4 and Table III). We finally obtained about 500 nmol of the purified fragment by repeating these steps.

Chemical Analyses of Carboxy-terminal Fragment—The purified fragment was hydrolyzed in 6 M HCl at 110 °C for 24 h and analyzed by a Hitachi amino acid analyzer (Model 835) as described under "Experimental Procedures." EnNH₂, ethanolamine.
The Gly content was variable compared with those of Asp and Thr depending upon the sample preparations. In addition, the fragment contained glucosamine and ethanolamine. The sample was also analyzed for other chemical compositions. All the compositions were normalized for their molar ratios by taking Asp as 1 mol, as summarized in Table III. In comparison with the values in Table II, it is evident that the purified fragment contains the same amounts of the characteristic components in sALP: 2 mol of ethanolamine, 1 mol of inositol, and 2 mol of phosphate. In addition, it contains glucosamine (1 mol) and mannose (3 mol), which are clearly different from the carboxyhydrate composition of its N-linked oligosaccharide chain (36).

The same sample was analyzed for its amino acid sequence, demonstrating that it has the tripeptide Thr-Thr-Asp, in good agreement with its composition (Table III). At the first cycle of Edman degradation, we always detected a significant amount of Gly. Since the composition analysis also indicates the presence of Gly, the purified sample is considered to contain the tetrapeptide Gly-Thr-Thr-Asp in part. The triterp peptide (Gly)-Thr-Thr-Asp is identified at positions (481)482-484 in the primary structure predicted from the cDNA sequence (6-8), as shown in Fig. 5. This sequence cannot be seen in any other part of the structure.

**DISCUSSION**

In this study, we selectively prepared mALP and sALP from human placental microsomes by 1-butanol extraction at pH 8.5 and 5.5, respectively. sALP showed the same mobility as the soluble form obtained by phosphatidylinositol-specific phospholipase C in polyacrylamide gel electrophoresis in the presence or absence of SDS. Such a differential preparation of mALP and sALP may be due to the presence of an endogenous phosphatidylinositol-specific phospholipase C-like enzyme which is activated at pH 5.5 in the presence of 1-butanol (12, 20, 21). In SDS-polyacrylamide gel electrophoresis, sALP migrated slightly slowly compared with mALP, the relationship of which was also observed for ALP from rat tissues (13, 37) and for the major glycoprotein from rat hepatoma plasma membranes (38). Thus, it seems likely that removal of a hydrophilic component from mALP retards the electrophoretic mobility in the presence of SDS. This is in contrast to the fact that another soluble form (psALP) prepared by papain digestion migrated slightly faster than mALP and sALP (Fig. 2B).

The relationship of mALP, sALP, and psALP was chemically examined (Table II). mALP contained palmitate, stearate, and glycerol, each at 1 mol/subunit, which may comprise a diacylglycerol moiety accounting for its hydrophobic nature. The presence of palmitate and/or stearate was also demonstrated in Thy-1 (17, 39) and acetylcholinesterase (18), whereas VSG of trypanosomes contained only myristate (16). The hydrophilicity of both sALP and psALP was confirmed by the absence of the lipid components. However, sALP still contained inositol and ethanolamine at the same amounts as those in mALP, whereas psALP contained neither. These results suggest that sALP is derived from mALP primarily by removal of the diacylglycerol moiety and is distinctly different from psALP. The presence of phosphate (1 mol) in psALP indicates that mALP and sALP actually contain 3 and 2 mol, respectively, of phosphate in site(s) other than the polypeptide remaining after papain digestion. The difference of the phosphate content between mALP and sALP suggests two possibilities. One is that sALP released by endogenous phosphatidylinositol-specific phospholipase C might initially contain 3 mol of phosphate, but lose 1 mol of phosphate with monoester linkage by its own phosphatase activity during the purification steps. The other is that sALP might be originally released from membranes with an endogenous or contaminating phospholipase D, as suggested by other investigators (40, 41).

Isolation of the membrane-anchoring domain of ALP has been done by preparing [3H]ethanolamine-labeled sALP as the starting material, followed by papain digestion. This is based on the following findings: sALP contains the characteristic components ethanolamine and inositol of the proposed anchor (14, 15), and the isolation of a hydrophilic component was found to be much easier than that of a hydrophobic one from [3H]palmitate-labeled mALP. For the first purification step, we employed immunoaffinity chromatography. As expected, most of the radioactivity flowed through the column, resulting in separation of the [3H]-labeled carboxyl-terminal fragment from the bulk portion of ALP which adsorbed to the column. ConA-Sepharose chromatography was also effective for removal of free [3H]ethanolamine and other components still contaminating the sample. Although placental ALP has a single N-linked oligosaccharide (7, 36), it should have been removed by the immunoaffinity chromatography. The specific binding of the [3H]-labeled fragment to ConA-Sepharose was supported by the presence of mammone residues in the fragment.

The final purified fragment contained the oligopeptide (Gly)-Thr-Thr-Asp, ethanolamine, mannose, glucosamine, inositol, and phosphate. On the premise that the inositol identified here would comprise a phosphatidylinositol moiety together with diacylglycerol found in mALP, a possible structure of the membrane-anchoring domain of placental ALP may be essentially the same as that proposed for VSG (16). There are some differences between our data and those previously reported (VSG of trypanosomes (16) contains ethanolamine (1 mol), galactose (2-4 mol), and myristic acid (2 mol); and Thy-1 (17) has galactosamine (1 mol)), although all the other components are the same as ours. Such differences possibly due to the species difference, however, may not influence the principal feature of the structure proposed: ethanolamine-glycan-phosphatidylinositol (14-16).

The sequence Gly-Thr-Thr-Asp of the purified fragment is identified at positions 491-494 of the primary structure deduced from the cDNA sequence (6-8). Thus, it is concluded that the actual mature form lacks the further carboxyl-terminal extension to position 513 which was predicted by the cDNA. The predicted peptide extension contains a stretch of 22 hydrophobic amino acids. It is proposed that primary structures of proteins to be anchored by the glycolipid, when predicted by the cDNAs, have such a hydrophobic carboxyl-terminal peptide domain, which is lost in the actual forms.
purified from cells (14, 15). However, there have been only a few cases in which the carboxyl-terminal amino acid in the mature protein is identified: VSG (14, 42), Thy-1 (17), human erythrocyte acetylcholinesterase (18), and human placental ALP (this study).

The glycosylphospholipid anchor of ALP has recently been characterized by labeling experiments (24, 43, 44). In a previous study (24) using JEG-3 cells, we demonstrated that placental ALP is initially synthesized as a precursor which is immediately converted to the modified form, resulting in the loss of about 1500 Da in its molecular mass. The precursor possibly corresponds to the form predicted by the cDNA. The loss in molecular mass during the conversion is reasonably accounted for by proteolytic removal of the carboxyl-terminal extension (29 amino acids) and by replacement with the glycosylphospholipid anchor at the newly formed carboxyl-terminal Asp.

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