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ISOLATION AND CHARACTERIZATION OF THE 36-kDa D-MANNOSE 6-PHOSPHATE RECEPTOR FROM PORCINE TESTIS

TADASHI BABA, KEN WATANABE, AND YUJI ARAI
Institute of Applied Biochemistry, University of Tsukuba, Ibaraki 305 (Japan)
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ABSTRACT

A D-mannose 6-phosphate receptor was isolated from total membranes of porcine testis, and its interaction with ligands was examined. The receptor was a glycoprotein comprised of several 36-kDa sub-units with an isoelectric point (pI) of 6.1. The binding of the receptor to the insoluble phosphomannan core occurred in the absence of divalent cations, but was selectively stimulated by $MnCl_2$ and effectively inhibited by D-mannose 6-phosphate, D-fructose 1-phosphate, and pentamannosyl monophosphate. The phosphate group and HO-2 of D-mannose 6-phosphate are important in the receptor–ligand interaction, HO-4 probably contributes to a lesser extent, and HO-1 seems to have no interaction.

INTRODUCTION

Lysosomal enzymes, of which high-mannose oligosaccharides are processed in the Golgi apparatus to generate the phosphomannosyl recognition marker, are targetted by the mannose 6-phosphate receptor (PMR)[1–3]. The function of PMR is the transport of these enzymes to the lysosome. PMRs have been purified from a variety of cells and tissues, and considered to be a monomeric protein with an apparent mass of 215 kDa[4–6]. A number of biochemical and histochemical studies have been reported[7–9].

Some cell lines are known to lack the 215-kDa PMR or to have a weak receptor activity[10,11]. However, the receptor-deficient cells possess high levels of intracellular acid-hydrolase activity, suggesting a presence of another form of PMR. A new PMR comprised of 46-kDa sub-units was recently isolated from murine $P388D_1$ macrophage and bovine liver[12], and was distinguishable from the 215-kDa receptor by its requirement for divalent cations and its low affinity for methyl phosphodiesters[12]. Although there are some reports[13–15] on PMRs comprised of several sub-units smaller than 215 kDa, these receptors bind lysosomal enzymes independent of divalent cations. Thus, the recognition system of lysosomal enzymes seems to involve multiple forms of PMRs. Therefore, it is necessary to isolate and characterize the low-molecular-weight PMRs.

We now report purification and characterization of PMR from porcine testis.

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EXPERIMENTAL

Materials. — *O*-Phosphomannan from *Hansenula holstii* was a generous gift from Dr. M. E. Slodki (U.S. Department of Agriculture, Peoria, Illinois). The phosphomannan core and pentamannosyl monophosphate were prepared as described previously[16]. The core fragment was coupled[12] to cyanogen bromide-activated Sepharose 4B, and the product contained 4 mg of phosphomannan core per mL of Sepharose. Fresh porcine testes were obtained from a local slaughterhouse, and their acetone powders were prepared as described previously[17]. Phosphorylated monosaccharides (sodium salts) were purchased from Sigma, and *Streptomyces griseus* endoglycosidase H (EC 3.2.1.96) and almond glycopeptidase A (EC 3.5.1.52) from Seikagaku Kogyo Co. Ltd. (Tokyo, Japan).

Purification of PMR. — The procedure was essentially that reported[12]. Briefly, a suspension of acetone powder (100 g) in 1 L of 50mM acetate buffer (pH 4.6) containing 0.15M NaCl and 0.5mM $CaCl_2$ was stirred for 1 h and centrifuged for 30 min at 16,000*g*. The pellet was washed with the same buffer (1 L), then with 50mM imidazole/HCl buffer (pH 7.0) containing 0.15M NaCl (buffer *A*) together with mM EDTA (1 L). The washed, membranous material was suspended in 1.5 L of buffer *A* containing 5mM sodium β-glycerophopshate, 1% of Triton X-100, 0.1% of sodium deoxycholate, and 15 mTIU/mL of Aprotinin (Sigma), stirred overnight, and then centrifuged. To the supernatant solution was added solid $MnCl_2$ to 10mM. After centrifugation for 60 min at 28,000*g*, the detergent-solubilized extract was passed through an affinity column (20-mL bed volume) of phosphomannan core–Sepharose 4B equilibrated with 50mM imidazole/HCl (pH 7.0) containing 0.15M NaCl and 0.05% of Triton X-100 (buffer *B*) together with 5mM sodium β-glycerophosphate and 10mM $MnCl_2$. The column was thoroughly washed with the equilibrating buffer, and proteins were eluted with the same buffer containing 2mM D-mannose 6-phosphate. The eluate was concentrated to 3 mL using a Mini-Module NM-3 ultrafiltration system (Asahi Kasei Ltd., Japan), and dialyzed overnight against 0.1M Hepes/NaOH (pH 7.0) containing 0.15M NaCl and 0.05% of Triton X-100 (buffer *C*). All purification procedures were carried out at 0–4°.

Iodination of PMR. — Purified proteins (100 μg) were iodinated on ice for 30 min with 0.5 mCi of $Na^{125}I$ (14 Ci/mg of iodine, Amersham Corp.) in a glass tube precoated with 50 μg of Iodogen (Pierce Chemical Co.). The iodinated proteins were filtered through a Sephadex G-25 column (Pharmacia, PD-10) equilibrated with buffer *C* containing KI (1 mg/mL) and BSA (1 mg/mL), and the excluded material was dialyzed against buffer *B* containing 5mM sodium β-glycerophosphate, 10mM $MnCl_2$, and BSA (0.5 mg/mL). The dialyzed solution was subjected to affinity chromatography on a 2-mL phosphomannan core–Sepharose column, as described above. The active, iodinated PMR was then filtered through a column of Sepharose CL-6B (see Fig. 1). A single, symmetrical peak was observed, and the peak fractions were combined, dialyzed against buffer *B* containing BSA (0.5 mg/mL), and used as the purified PMR. There was no significant loss of binding activity during storage at 4° for 4 weeks.

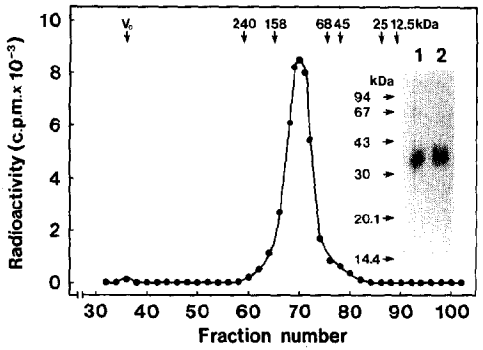


Fig. 1. Gel filtration of iodinated PMR on Sepharose CL-6B. The active, iodinated PMR (4 mL) was applied to a column (1.9 × 95 cm) of Sepharose CL-6B previously equilibrated with buffer *B* containing BSA (0.5 mg/mL), and eluted with the same buffer at 4°. Fractions (2.6 mL) were collected at 10 mL/h. Catalase (240 kDa), aldolase (158 kDa), BSA (68 kDa), ovalbumin (45 kDa), chymotrypsinogen A (25 kDa), and cytochrome *c* (12.5 kDa) were used as molecular weight standards. Inset: SDS-PAGE (10% polyacrylamide gel) of the purified PMR under non-reducing (lane 1) and reducing conditions (1% of 2-mercaptoethanol) (lane 2); autoradiography was carried out on the dried gel. The masses (kDa) indicated represent the following standards (Pharmacia): phosphorylase b, 94; BSA, 67; ovalbumin, 43; carbonic anhydrase, 30; trypsin inhibitor, 20.1; α-lactalbumin, 14.4.

Binding assay for PMR. — The binding activity of iodinated PMR was measured by incubating the receptor with phosphomannan core–Sepharose 4B. The reaction mixture (100 μL) consisted of 20,000 c.p.m. of iodinated PMR and the insoluble phosphomannan core (35 μL of gel) in buffer *B* containing BSA (0.5 mg/mL). The reaction was carried out for 90 min at room temperature in a 1.5-mL centrifuge tube. After centrifugation in an Eppendorf model 5414 microcentrifuge, an aliquot (50 μL) of the supernatant solution was measured for radioactivity, using a Beckman gamma 9000 counter. Of the iodinated PMR added, ~70% was bound to the ligands in the presence of 10mM $MnCl_2$. All experimental data were obtained at least in triplicate, and the control mixture contained 5mM D-mannose 6-phosphate or unsubstituted Sepharose 4B instead of the insoluble phosphomannan core.

Deglycosylation of iodinated PMR. — The iodinated PMR (200,000 c.p.m.) was incubated for 16 h at 37° in a mixture (1 mL) of 0.1M citrate/phosphate buffer (pH 5.0), mM phenylmethylsulfonyl fluoride, 0.01mM pepstatin A, and endoglycosidase H or glycopeptidase A (0.5 mU of each). The deglycosylated protein was analyzed by gel filtration on a column (1.2 × 90 cm) of Sephacryl S-200 which had been equilibrated with 50mM Hepes/NaOH (pH 7.0) containing 0.15M NaCl, 0.05% of Triton X-100, and BSA (0.5 mg/mL). Fractions (1 mL) were collected at 5 mL/h. A portion (0.5 mL) of each fraction was measured for radioactivity, as described above. Blue dextran, aldolase (158 kDa), BSA (68 kDa), ovalbumin (45 kDa), chymotrypsinogen A (25 kDa), and cytochrome *c* (12.5 kDa) were used as standards.

Analytical procedures. — Proteins were determined by the method of Bradford[18], using BSA as a standard. Polyacrylamide gel electrophoresis (6.0, 7.5,

or 10.0% acrylamide gel) in the presence of sodium dodecyl sulfate (SDS-PAGE) was carried out by the method of Laemmli[19]. Isoelectric focusing of iodinated PMR was performed on a horizontal slab gel (4.85% acrylamide) with 2.67% Ampholine (pH 3.5–10.0, LKB), using a Resolmax-IEF apparatus (ATTO Corp., Japan). After electrophoresis at 200 V for 3 h, the gel was cut into pieces (1.0 × 0.5 cm) and each piece was measured for pH and radioactivity.

RESULTS

By affinity chromatography on a phosphomannan core–Sepharose 4B column, 900 μg of proteins were purified from 100 g of porcine testis acetone powder and found to contain two different proteins which migrated on SDS-PAGE with sub-unit masses of 230 and 36 kDa (not shown). The 36-kDa sub-unit gave a major but a relatively diffuse band. When the two purified proteins were iodinated together with $Na^{125}I$, re-purified on the same affinity column, and then filtered through a Sepharose CL-6B column in the presence of 0.05% Triton X-100, the active, iodinated PMR was eluted as a single symmetrical peak in the position of 118 kDa (Fig. 1). Analysis of the isolated PMR on SDS-PAGE under reducing or non-reducing conditions revealed that the receptor had a sub-unit mass of 36 kDa, although a 72-kDa fragment was detected as a very minor band under the non-reducing conditions (Fig. 1, inset).

When the iodinated PMR was treated with endoglycosidase H or glycopeptidase A, the mass of the deglycosylated sub-units by either treatment was estimated to be 33 kDa on SDS-PAGE (Fig. 2). The elution profiles of the deglycosylated PMRs on Sephacryl S-200 indicated that the receptors treated with endoglycosidase H and glycopeptidase A had molecular weights of 115,000 and 111,000, respectively (not shown).

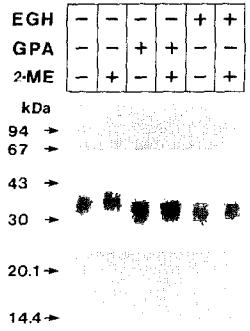


Fig. 2. Treatments of iodinated PMR with (+) or without (−) endoglycosidase H (EGH) and glycopeptidase A (GPA) (see Experimental). A portion (~10,000 c.p.m.) was subjected to SDS-PAGE (10% acrylamide gel) under reducing or non-reducing conditions [1% of 2-mercaptoethanol (2-ME)]. The ^{125}I-labeled compounds were detected by autoradiography.

TABLE I

EFFECTS OF CATIONS AND EDTA ON BINDING OF 36-kDa PMR TO INSOLUBLE PHOSPHOMANNAN CORE

Compound	*Relative binding activity*[a] *(%)*	*Compound*	*Relative binding activity*[a] *(%)*
10mM $MnCl_2$	100	5mM $FeSO_4$	81 ±3
None	46 ±4	$MgCl_2$	26 ±5
5mM KCl	28 ±6	$MnCl_2$	83 ±1
LiCl	19 ±8	$NiCl_2$	41 ±4
$CaCl_2$	54 ±5	$ZnCl_2$	6 ±4
$CdCl_2$	17 ±1	$AlCl_3$	140 ±1
$CoCl_2$	59 ±1	$FeCl_3$	150 ±1
$CuCl_2$	14 ±3	EDTA	58 ±2

[a]The binding activity of iodinated 36-kDa PMR was measured under the standard assay conditions (in triplicate) in the absence or presence of various cations and EDTA (see Experimental).

The isoelectric focusing of iodinated PMR gave a pI of 6.1 (not shown). This value was relatively similar to those of two forms of PMR, with masses of 215 kDa, from Swarm rat chondrosarcoma membranes (pI 5.5 and 6.2)[7].

Table I shows that the iodinated 36-kDa PMR binds insoluble phosphomannan core in the absence of any cations, but the binding activity is nearly doubled by the presence of 10mM $MnCl_2$; $CaCl_2$, $CoCl_2$, $NiCl_2$, and EDTA at 5mM have no effect. KCl, LiCl, $CdCl_2$, $CuCl_2$, and $MgCl_2$ caused slight inhibition, $ZnCl_2$ caused considerable inhibition, and $FeSO_4$, $AlCl_3$, and $FeCl_3$ enhanced the binding but caused precipitation. The $MnCl_2$-stimulated binding reached a maximum at 10mM. In the presence of 10mM $MnCl_2$, *N*-ethylmaleimide (mM), *p*-chloromercuribenzoate (0.1mM), dithiothreitol (5mM), dithioerythritol (5mM), and 2-mercaptoethanol (5mM) did not have a significant effect on the ligand–PMR binding, whereas it was stimulated slightly by mM *o*-phenanthroline (not shown).

In the presence of 10mM $MnCl_2$, the binding of 36-kDa PMR to ligands was

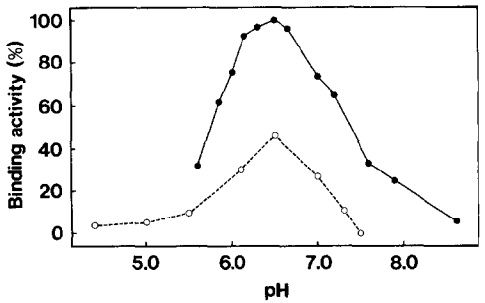


Fig. 3. Effect of pH on binding of PMR to insoluble phosphomannan core. The iodinated PMR (20,000 c.p.m.) was incubated at room temperature for 90 min with insoluble phosphomannan core (35 μL of gel) in 0.1M citrate/phosphate (···○···) and imidazole/HCl (—●—) buffers (100 μL) at various pH values. The binding activity was measured as described in the Experimental.

TABLE II

INHIBITION OF BINDING OF 36-kDa PMR TO INSOLUBLE PHOSPHOMANNAN CORE

Inhibitor (5 mM)	*Inhibition*[a] *(%)*
D-Mannose	7 ±3
D-Glucose	3 ±2
D-Galactose	2 ±1
D-Fucose	2 ±2
L-Fucose	7 ±1
L-Rhamnose	2 ±2
D-Mannitol	1 ±1
2-Amino-2-deoxy-D-mannose	0 ±1
2-Amino-2-deoxy-D-glucose	3 ±2
Methyl α-D-mannopyranoside	2 ±2
D-Mannose 6-phosphate	100 ±5
D-Glucose 6-phosphate	29 ±3
D-Galactose 6-phosphate	16 ±5
D-Fructose 6-phosphate	15 ±1
2-Amino-2-deoxy-D-glucose 6-phosphate	18 ±9
D-Mannopyranose 1-phosphate	15 ±7
D-Fructopyranose 1-phosphate	97 ±2
D-Ribose 5-phosphate	9 ±5
Pentamannosyl monophosphate	100 ±5

[a]In the presence of 10mM $MnCl_2$ under the standard assay conditions (in triplicate; see Experimental).

maximal at pH 6.5, and more than 75% of the maximal binding activity was found between pH 6.0 and 7.0 (Fig. 3). The binding activity was diminished by 50% of the maximum at pH 5.7 and 7.4, so that little activity was detectable at pH 5.0 and 8.5.

D-Mannose 6-phosphate, D-fructose 1-phosphate, and pentamannosyl monophosphate (5mM each, Table II) were the most effective inhibitors of the binding of PMR to the insoluble phosphomannan core, D-glucose 6-phosphate was a poor inhibitor, and D-galactose 6-phosphate, D-fructose 6-phosphate, 2-amino-2-

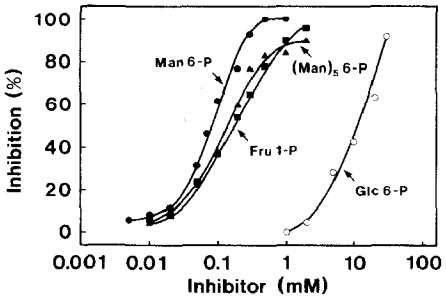


Fig. 4. Inhibition of binding of PMR to insoluble phosphomannan core by D-mannose 6-phosphate (—●—), D-fructose 1-phosphate (—■—), pentamannosyl monophosphate (—▲—), and D-glucose 6-phosphate (—○—). All assays were carried out in triplicate in the presence of 10mM $MnCl_2$ (see Table II).

deoxy-D-glucose 6-phosphate, D-mannose 1-phosphate, and D-ribose 5-phosphate were inactive as were deoxy and aminodeoxy sugars.

Fig. 4 shows that the binding activity of 36-kDa PMR is best inhibited by D-mannose 6-phosphate, with 50% inhibition at 0.08mM. Pentamannosyl monophosphate and D-fructose 1-phosphate gave 50% inhibitions at 0.15 and 0.17mM, respectively. D-Glucose 6-phosphate caused 43% inhibition at 10mM.

DISCUSSION

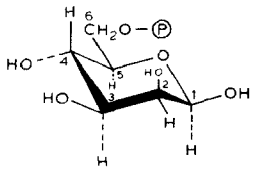
Evidence is presented for the existence of a low-molecular-weight PMR in porcine testis membranes. Two different proteins composed of 230- and 36-kDa sub-units were purified by phosphomannan core–Sepharose 4B affinity chromatography. The 230-kDa protein may correspond to 215-kDa PMR isolated by many workers[4–7]. The active, iodinated PMR was comprised only of 36-kDa sub-units (Fig. 1), of which the mass was 10 kDa less than that of a cation-dependent PMR from bovine liver[12]. This finding implied the loss of the ligand-binding activity of 230-kDa protein during the iodination. The digestion of 36-kDa PMR with endoglycosidase H or glycopeptidase A suggests that the receptor has some asparagine-linked oligosaccharides (Fig. 2). Based on the results of gel filtation on Sepharose CL-6B or Sephacryl S-200, the isolated PMR is characterized as a glycoprotein comprised of several 36-kDa sub-units. Since the same size of the sub-unit is found on SDS-PAGE under either reducing or non-reducing condition (Fig. 1), the sub-units are not associated by disulfide bonds.

The isolated 36-kDa PMR differs from the already purified 215-kDa receptor by having a sub-unit of smaller mass (Fig. 1) and binding activity stimulated selectively by $MnCl_2$ (Table I). Hoflack and Kornfeld[12] reported that a cation-dependent 46-kDa PMR from bovine liver efficiently bound insoluble phosphomannan core in the presence of 10mM $MnCl_2$, whereas there was only a weak interaction of the receptor and the ligands when EDTA was present. The binding of 36-kDa PMR to the same ligands was enhanced by $MnCl_2$, but EDTA or other divalent cations had no significant effect (Table I). Some divalent cations acted as inhibitors. Thus, the 36-kDa PMR may be a cation-independent receptor, but its properties seem to be similar to those of the cation-dependent 46-kDa PMR[12].

Distler and Jourdian[14,15] reported the isolation and characterization of a novel PMR-like protein composed of 42-kDa sub-units from bovine testes. The binding to β-D-galactosidase was not increased by 10mM $MnCl_2$ in contrast to our 36-kDa PMR. This difference may be because the insoluble phosphomannan core was used as the ligand, whereas Distler and Jourdian used β-D-galactosidase[14,15]. The low-molecular-weight PMRs from testicular tissues have sub-unit masses that are 4–10 kDa less than those from the other tissues.

D-Mannose 6-phosphate, D-fructose 1-phosphate, and pentamannosyl monophosphate were the best inhibitors for the 36-kDa PMR (Table II and Fig. 4). D-Mannopyranose and D-fructopyranose adopt $^4C_1$ and $^1C_4$ conformations[20],

respectively, and D-mannose 6-phosphate (**1**) and D-fructose 1-phosphate (**2**) should adopt these conformations. When the $^1C_4$ conformer of D-fructose 1-phosphate (**2**) is rotated[21] 180° around an axis through C-4 and O-6, HO-3,4,5 and the phosphate group are superimposable on the corresponding groups of D-mannose 6-phosphate, and both **1** and **2** are permitted as ligands.



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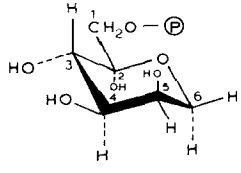

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The phosphate group of D-mannose 6-phosphate is probably most important for the PMR–ligand interaction, since D-mannose is not an inhibitor (Table II). Moreover, D-mannose 1-phosphate is a poor inhibitor. D-Glucose 6-phosphate (HO-2 equatorial) is a weaker inhibitor than D-mannose 6-phosphate (HO-2 axial) but a slightly better inhibitor than D-galactose 6-phosphate (HO-2 equatorial, HO-4 axial). Thus, it appears that the 36-kDa PMR prefers an axial HO-2 and that the interaction of equatorial HO-4 is effective but very weak. The HO-1 of D-mannose 6-phosphate is not important for the PMR–ligand interaction, since pentamannosyl monophosphate, α-D-Man-6-phosphate-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→2)-D-Man, is one of the best inhibitors. The importance of an equatorial HO-3 remains to be clarified.

The uptake study of lysosomal enzymes by human skin fibroblasts has been reported[22,23]. D-Mannose 6-phosphate and D-fructose 1-phosphate markedly inhibited the PMR-mediated uptake of the lysosomal enzymes, whereas D-glucose 6-phosphate and D-galactose 6-phosphate were poor inhibitors[22,23]. The results are consistent with the ligand-binding specificity of our 36-kDa PMR. The internalization of lysosomal enzymes with the PMR recognition marker presumably occurs only *via* the 215-kDa receptor, since there is no evidence that the low-molecular-weight PMRs are present at the cell surface[24]. It appears that the ligand-binding specificity between the 215- and 36-kDa PMRs is similar.

When this work was complete, a report appeared[25] on the interaction of phosphorylated oligosaccharides and lysosomal enzymes with bovine liver cation-dependent 46-kDa PMR. The binding of an oligosaccharide with one phosphomonoester to the 46-kDa PMR was optimal between pH 6.0 and 6.3. When the pH was raised to 6.9 and above, the interaction became weaker, and at pH 5.3 and below, there was no interaction. Therefore, our 36-kDa PMR binds the ligands over a slightly broader pH range than does the cation-dependent 46-kDa receptor (Fig. 3). Also, reports on mannose 6-phosphate receptors appeared[26–32] after this paper was submitted.

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REFERENCES

1 E. F. NEUFELD AND G. ASHWELL, in W. J. LENNARTZ (Ed.), *The Biochemistry of Glycoproteins and Proteoglycans*, Plenum, New York, 1980, pp. 241–266.
2 W. S. SLY AND H. D. FISCHER, *J. Cell Biochem.*, 18 (1982) 67–85.
3 G. G. SAHAGIAN, *Biol. Cell*, 51 (1984) 207–214.
4 G. G. SAHAGIAN, J. DISTLER, AND G. W. JOURDIAN, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 4289–4293.
5 G. G. SAHAGIAN AND E. F. NEUFELD, *J. Biol. Chem.*, 258 (1983) 7121–7128.
6 W. J. BROWN AND M. G. FARQUHAR, *Cell*, 36 (1984) 295–307.
7 A. W. STEINER AND L. H. ROME, *Arch. Biochem. Biophys.*, 214 (1982) 681–687.
8 G. G. SAHAGIAN AND C. J. STEER, *J. Biol. Chem.*, 260 (1985) 9838–9842.
9 W. J. BROWN, J. GOODHOUSE, AND M. G. FARQUHAR, *J. Cell Biol.*, 103 (1986) 1235–1247.
10 C. A. GABEL, D. E. GOLDBERG, AND S. KORNFELD, *Proc. Natl. Acad. Sci. U.S.A.*, 80 (1983) 775–779.
11 B. HOFLACK AND S. KORNFELD, *Proc. Natl. Acad. Sci. U.S.A.*, 82 (1985) 4428–4432.
12 B. HOFLACK AND S. KORNFELD, *J. Biol. Chem.*, 260 (1985) 12008–12014.
13 D. C. MITCHELL, T. MALER, AND G. W. JOURDIAN, *J. Cell Biochem.*, 24 (1984) 319–330.
14 J. DISTLER AND G. W. JOURDIAN, *Fed. Proc.*, 44 (1985) 1435.
15 J. DISTLER AND G. W. JOURDIAN, *Fed. Proc.*, 45 (1986) 1900.
16 R. K. BRETTHAUER, G. J. KACZOROWSKI, AND M. J. WEISE, *Biochemistry*, 12 (1973) 1251–1256.
17 G. G. SAHAGIAN, J. J. DISTLER, AND G. W. JOURDIAN, *Methods Enzymol.*, 83 (1982) 392–396.
18 M. M. BRADFORD, *Anal. Biochem.*, 72 (1976) 248–254.
19 U. K. LAEMMLI, *Nature (London)*, 227 (1970) 680–685.
20 S. J. ANGYAL, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates: Chemistry and Biochemistry*, Vol. 1A, Academic Press, Orlando, 1972, pp. 195–215.
21 R. S. HALTIWANGER AND R. L. HILL, *J. Biol. Chem.*, 261 (1986) 15696–15702.
22 A. KAPLAN, D. T. ACHORD, AND W. S. SLY, *Proc. Natl. Acad. Sci. U.S.A.*, 74 (1977) 2026–2030.
23 G. N. SANDO AND E. F. NEUFELD, *Cell*, 12 (1977) 619–627.
24 C. A. GABEL AND S. A. FOSTER, *J. Cell Biochem.*, 103 (1986) 1817–1827.
25 B. HOFLACK, K. FUJIMOTO, AND S. KORNFELD, *J. Biol. Chem.*, 262 (1987) 123–129.
26 P. LOBEL, N. M. DAHMS, J. BREITMEYER, J. M. CHIRGWIN, AND S. KORNFELD, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 2233–2237.
27 N. M. DAHMS, P. LOBEL, J. BREITMEYER, J. M. CHIRGWIN, AND S. KORNFELD, *Cell*, 50 (1987) 181–192.
28 R. POHLMANN, G. NAGEL, B. SCHMIDT, M. STEIN, G. LORKOWSKI, C. KRENTLER, J. CULLY, H. E. MEYER, K. GRZESCHIK, G. MERSMANN, A. HASILIK, AND K. VON FIGURA, *Proc. Natl. Acad. Sci. U.S.A.*, 84 (1987) 5575–5579.
29 M. STEIN, J. E. ZIJDERHAND-BLEEKEMOLEN, H. J. GEUZE, A. HASILIK, AND K. VON FIGURA, *EMBO J.*, 6 (1987) 2677–2681.
30 M. STEIN, H. E. MEYER, A. HASILIK, AND K. VON FIGURA, *Biol. Chem. Hoppe-Seyler*, 368 (1987) 927–936.
31 M. STEIN, T. BRAULKE, C. KRENTLER, A. HASILIK, AND K. VON FIGURA, *Biol. Chem. Hoppe-Seyler*, 368 (1987) 937–947.
32 D. O. MORGAN, J. C. EDMAN, D. N. STANDRING, V. A. FRIED, M. C. SMITH, R. A. ROTH, AND W. J. RUTTER, *Nature (London)*, 329 (1987) 301–307.