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Solubilization and reconstitution of hepatic System A-mediated amino acid transport. Preparation of proteoliposomes containing glucagon-stimulated transport activity

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System A-mediated amino acid transport activity from rat liver plasma membrane vesicles has been solubilized and reconstituted into proteoliposomes using a freeze-thaw-dilution technique. The presence of cholate, at a cholate to protein ratio of 1:1, during the freeze-thaw step resulted in an enhancement in recoverable transport activity. The carrier required both phosphatidylcholine and phosphatidylethanolamine for optimal activity, but the addition of cholesterol to the reconstitution procedure appeared to have no significant effect on the resulting activity. A lipid to protein ratio of 20:1 yielded maximal transport activity. Sonication of the proteoliposomes provided some improvement in the accuracy of replicate assays for a given proteoliposome preparation. Isolated liver plasma membrane vesicles prepared from rats treated in vivo with glucagon in combination with dexamethasone contained stimulated System A activity. This enhanced transport activity could be solubilized and recovered in proteoliposomes generated from these plasma membranes. The data support the proposal that hormone regulation of the hepatic System A gene results in the de novo synthesis and plasma membrane insertion of the carrier protein itself.

Introduction

The basal activity of hepatic amino acid transport mediated by System A can be stimulated by either hormones or substrate starvation (for reviews, see Refs. 1 and 2). Experiments in both normal hepatocytes and several hepatoma cell lines have shown that the induction is blocked by macromolecular synthesis inhibitors for total RNA [3,4], poly(A)$^+$ mRNA [5,6], total protein [4,7], or asparagine-linked glycoproteins [4,8]. Collectively, these results demonstrate that increased transcription of one or more genes results in the de novo synthesis of a System A-associated glycoprotein. Although it is not known whether the newly synthesized protein represents the plasma membrane carrier itself, the presence of carbohydrate sidechains is consistent with that hypothesis. As further evidence for this proposal, stimulated System A activity is retained in rat liver membrane vesicles prepared from whole livers of fasted [9], diabetic [10] or glucagon-treated animals [11], or from isolated hepatocytes exposed to cAMP [12].

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Abbreviations: PMSF, phenylmethylsulfonyl fluoride; $C_{12}E_9$, polyoxyethylene-9-lauryl ether; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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System A transport activity has been solubilized and reconstituted from mouse fibroblast [13] and Ehrlich ascites tumor cell [14–16] plasma membranes. For further studies on the reconstitution of System A, the choice of liver tissue appears to be a favorable one. Large amounts of a plasma membrane-enriched fraction can be prepared from either rat or bovine liver, and in the case of the rat tissue the activity can be induced by 20-fold or more through the use of hormone treatment [17]. Plasma membrane vesicles containing either basal or glucagon-induced System A activity have been used as the starting material for the reconstitution studies reported here. System A activity can be solubilized from these membrane vesicles through the use of either non-ionic or ionic detergents and then reconstituted into proteoliposomes by a freeze-thaw-dilution technique [18] that has been modified to include cholate [19,20].

Materials and Methods

Preparation of rat liver plasma membrane vesicles

A plasma membrane-enriched fraction was prepared from intact livers taken from either normal or glucagon-injected rats by a modification of the method described by Prpic et al. [21]. Unless indicated otherwise, male Sprague-Dawley rats, weighing 150–200 g, were injected with 1 mg glucagon per 100 g body weight 4–6 h prior to plasma membrane isolation. After anesthetizing the animal, the liver was perfused for 10 min with ice-cold phosphate-buffered saline (PBS: 10 mM sodium phosphate/150 mM sodium chloride (pH 7.4)). The liver was removed, weighed (5–8 g), and placed in an equal volume (w/v) of buffer A/EGTA (250 mM sucrose/1 mM EGTA/10 mM Tris (pH 7.5)). The liver was homogenized using a glass Dounce homogenizer by 10 strokes with a loose-fitting pestle followed by 4 strokes with a tight-fitting pestle. The homogenate was diluted to 6% (w/v) with Buffer A/EGTA based on the original number of grams of tissue used and then centrifuged at $150 \times g$ for 2 min. The pellet was discarded and the supernatant was centrifuged at $1\,500 \times g$ for 10 min at 4°C in a Sorvall SS-34 rotor. The resulting supernatant was discarded and the pellet resuspended in a total volume of 30 ml of Buffer A/EGTA. The suspension was filtered through cheese-cloth, brought to a final volume of 31.2 ml with Buffer A/EGTA, and 4.2 ml of Percoll was added. After mixing thoroughly, 11.8 ml was transferred into each of three 15 ml Corex centrifugation tubes and the suspension was centrifuged at $34\,500 \times g$ for 30 min at 4°C in a Sorvall SS-34 rotor. A layer of lipid that floated on top of the gradient was removed by aspiration and the next band of material, containing the plasma membrane-enriched fraction, was removed using an 18 gauge needle attached to a syringe. The membranes were diluted 1 : 6 with Buffer A (250 mM sucrose/10 mM Tris (pH 7.5)) and centrifuged at $34\,500 \times g$ for 30 min at 4°C in a Sorvall SS-34 rotor. The resulting supernatant was removed carefully by aspiration and the pellet resuspended in Buffer A to a concentration of approx. 10 mg/ml. Aliquots were taken from the freshly isolated membranes to measure protein content and to assay the System A transport activity as described below. The remainder of the membrane vesicle preparation was divided into 50-µl aliquots and stored frozen at −75°C. System A transport activity was sensitive to repeated cycles of thawing, so each aliquot was thawed only once.

Detergent-extraction of rat liver plasma membranes

Membrane proteins were extracted with detergent by the method of McCormick et al. [16]. Vesicles were diluted into solubilization buffer (5 mM Hepes (pH 7.5)/100 mM KCl/4 M urea/2.5% cholate/0.1 mM EDTA/1 mM PMSF) at a final concentration of approx. 2 mg/ml. After incubation of the mixture for 30 min at room temperature, the insoluble membrane remnants were pelleted by centrifugation at $100\,000 \times g$ for 45 min. After removing an aliquot for determination of total protein, the supernatant was dialyzed for 18 h (overnight) against a buffer containing 10 mM Hepes (pH 7.5)/0.2% $C_{12}E_9$/10 mM $MgCl_2$/100 mM KCl/1 mM PMSF). The dialyzed extract was divided into 50-µl aliquots and then stored frozen at −75°C. The System A activity in the frozen extract was stable for several weeks. If the 2.5% cholate present in the solubilization buffer was replaced by 2% $C_{12}E_9$, similar results were obtained with regard to the absolute velocity of System A activity in the proteolipo-

somes. However, cholate proved to be more efficient in solubilizing the System A carrier protein (78% versus 47%) and was used routinely.

The protein content of the samples (vesicles or detergent extracts) was measured after trichloroacetic acid precipitation of the protein and resuspension in 0.2 M NaOH containing 0.2% sodium dodecyl sulfate (SDS). A copper reagent containing 0.58 mM EDTA (copper-disodium salt), 189 mM $NaCO_3$, 100 mM NaOH, and 1% SDS [22,23] was added at a ratio of 600 μl copper reagent for each 100 μl of protein sample. After incubation of the samples for 10 min at room temperature, 60 μl of Folin-Ciocalteau reagent (diluted with water 1 : 1 just before use) was added to each sample. The absorbance of each sample and a bovine serum albumin standard curve was measured at 750 nm.

Freeze-thaw reconstitution procedure

The following outline of the reconstitution procedure represents the optimum conditions as determined in the experiments presented in Results. A stock solution (40 mg/ml) of asolectin (soybean phospholipid) was prepared by suspending the lipid in 10 mM Hepes (pH 7.5)/200 mM KCl/1 mM $MgCl_2$ (K^+-uptake buffer) and sonicating the mixture in a bath-type sonicator until a clear solution was obtained. Reconstitution of System A activity was performed by mixing 1 mg of solubilized membrane protein, 20 mg of the sonicated asolectin, and 1 mg of recrystallized cholate (potassium salt, the stock solution was 10% w/v). The solution, approx. 1 ml total volume, was frozen in liquid nitrogen, thawed at room temperature, and then diluted with 2 ml of K^+-uptake buffer. The reconstituted proteoliposomes were then sonicated for 20 s using a bath-type sonicator. The mixture was centrifuged at 125000 × *g* for 45 min at 4° C and the resulting proteoliposome pellet was resuspended in 200 μl of K^+-uptake buffer with gentle vortexing.

Amino acid transport assay

The transport assays were initiated by adding 20 μl (about 20 μg membrane protein) of either plasma membrane vesicles or reconstituted proteoliposomes to 20 μl of a solution containing 200 μM ^{3}H-labeled 2-aminoisobutyric acid (0.5 μCi per assay) in K^+- or Na^+-uptake buffer (10 mM Hepes (pH 7.5), 200 mM KCl or NaCl and 1 mM $MgCl_2$). The mixture was vortexed and incubated at 22° C for the time indicated in the legend to each figure or table. The uptake was terminated by the addition of 1 ml of ice-cold stop buffer (10 mM sodium phosphate (pH 7.5)/150 mM sodium chloride) and the suspension was immediately passed over a nitrocellulose filter (0.45 μm). The filter was washed once with 3 ml of ice-cold stop buffer, placed into a scintillation vial, and then 5 ml of Bray's scintillation cocktail [24] was added to allow determination of the trapped radioactivity. Non-specific binding of the ^{3}H-labeled 2-aminoisobutyric acid to the nitrocellulose filters was measured and subtracted from all assays. The data presented are typically from a single membrane preparation, although each experiment has been repeated using at least two different membrane preparations to ensure that the results were reproducible qualitatively.

Materials

Cholic acid and purified phospholipids were obtained from Sigma Chemical Company. The cholic acid was recystallized three times in ethanol as described by Kagawa and Racker [25]. Ultrapure urea was purchased from Pierce Chemicals and asolectin was obtained from Associated Concentrates Inc. (Woodside, N.Y.). The 2-amino-iso[*methyl*-^{3}H]butyric acid (AIB) was purchased from ICN Pharmaceuticals. Highly purified glucagon was a generous gift from Dr. William W. Bromer of Lilly Laboratories. Nitrocellulose filters used for the transport assays were either Millipore Type HAWP or Gelman Type GN-6 (0.45 μm). Male Sprague-Dawley rats were obtained from a colony maintained by the University of Florida Animal Resources Facility.

Results

Effects of lipid on the reconstitution of hepatic System A

Previous reconstitutions of a number of membrane-bound enzymes and transport proteins have shown that the amount of lipid present during the formation of the proteoliposomes can influence the recovery of activity [26]. The lipid (asolectin)

to protein ratio of the freeze-thaw mixture during reconstitution of hepatic System A from glucagon-treated rats was varied from 2 : 1 to 30 : 1 (w/w). The protein concentration was held constant at approx. 1 mg/ml and the cholate to protein ratio was maintained at 1 : 1 (w/w). The data presented in Fig. 1 illustrate the results of such an experiment. In the absence of added lipid, no measurable Na^+-dependent transport activity is recovered demonstrating that no intact membrane vesicles remain in the solubilized protein fraction under study. The System A activity increased in a nearly linear manner between lipid to protein ratios of 2 : 1 and 20 : 1. At ratios greater than 20 : 1, the activity obtained was variable between experiments. In general, a slight decrease in transport activity from that obtained at a ratio of 20 : 1 was seen when ratios of 30 : 1 to 50 : 1 were tested (data not shown).

In addition to the amount of lipid, the composition of the lipid plays an important role in determining the amount of activity recovered. It is apparent that individual membrane-bound enzymes or transporters can respond to compositional changes in the lipid environment [27]. For the reconstitution of hepatic System A, replacement of asolectin, a mixture of soybean phospholipids, by either phosphatidylcholine or phosphatidylethanolamine alone resulted in little or no measurable activity (velocities were 166 ± 101 and 130 ± 102 pmol · (mg protein)$^{-1}$ · min^{-1} for phosphatidylcholine and phosphatidylethanolamine, respectively). In contrast, a mixture of phosphatidylcholine and phosphatidylethanolamine was able to support reconstitution at rates similar to those obtained with asolectin (velocities were 707 ± 202 and 715 ± 112 pmol · (mg protein)$^{-1}$ · min^{-1} for the mixture and asolectin, respectively). Based on these studies, all remaining experiments were performed with asolectin at a lipid to protein ratio of 20 : 1.

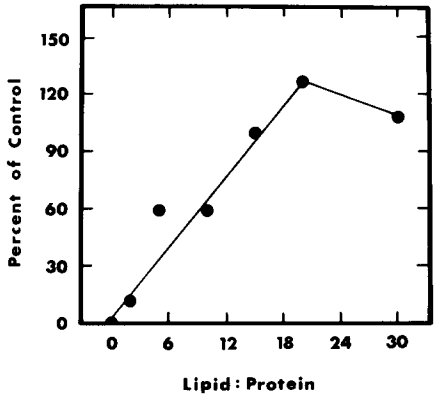

Fig. 1. Titration of the lipid to protein ratio for reconstitution of System A activity from rat liver. Solubilized membrane protein was reconstituted in the presence of the indicated amounts of sonicated asolectin. All of the other variables were set at the optima indicated in Methods and Materials. System A activity was assayed by measuring the Na^+-dependent uptake of 200 μM 2-aminoisobutyric acid (AIB) for 1 min at room temperature. The data are expressed as the percent of the value obtained at a lipid to protein ratio of 15 : 1 which was 489 ± 29 pmol AIB · (mg protein)$^{-1}$ · min^{-1}. In the absence of added lipid (lipid to protein ratio of 0 : 1), no detectable activity was observed following reconstitution. The standard deviations for assays in quadruplicate were typically less than 10%.

The plasma membrane of the rat hepatocyte contains a significant amount of cholesterol, a lipid component that is required during the reconstitution of the acetylcholine receptor to regain full biological activity [27]. To determine the influence of cholesterol on the activity of reconstituted System A, the freeze-thaw step was performed using a lipid to protein ratio of 20 : 1 and a portion of the asolectin was replaced with cholesterol, such that cholesterol represented 0, 10, 15 or 20% of the total lipid present. The velocities of System A obtained following reconstitution under these conditions were not dramatically different regardless of the cholesterol content of the freeze-thaw mixture (velocities were 740 ± 170, 882 ± 95, 773 ± 160, and 625 ± 50 pmol · (mg protein)$^{-1}$ · min^{-1} at a cholesterol percentage of 0, 10, 15, and 20%, respectively).

Effect of detergent on the reconstitution of System A

Detergent is an important component of the outlined reconstitution procedure at two different steps. Although non-ionic detergents such as $C_{12}E_9$ can be employed during solubilization, typically, cholate was used to yield a soluble membrane-protein fraction. In addition, it has been reported that the presence of cholate in the mixture that is subjected to the freeze-thaw cycle is beneficial for the reconstitution of the $(Na^+ + K^+)$-ATPase [19] and the mitochondrial proton-pump [20]. In our hands, a similar addition allowed recovery of the

maximal System A activity from membranes isolated from Ehrlich ascites tumor cells [28]. For example, reconstitution of Ehrlich cell plasma membrane proteins in the absence of cholate during the freeze-thaw step resulted in Na^+-dependent 2-aminoisobutyric acid transport of approx. 300 pmol · (mg protein)$^{-1}$ · min^{-1}, whereas in the presence of an optimal amount of cholate the transport rate was nearly 1 200 pmol · (mg protein)$^{-1}$ · min^{-1}. To determine whether cholate had an effect on the reconstituted System A activity from rat liver, we measured the transport by proteoliposomes prepared with varying amounts of cholate present during the freeze-thaw cycle. Although the effect of added cholate for this tissue was not as pronounced as that seen for the activity from Ehrlich cells, at cholate to protein ratios of 1 : 1 or 2 : 1 there was a substantial increase in the amount of System A-mediated transport (Fig. 2). In addition, it was noted that the reproducibility within each experiment and between experiments was enhanced by the inclusion of the cholate. For all other studies described in this report, each of the freeze-thaw cycles were performed with a cholate to protein ratio of 1 : 1.

Effect of proteoliposome sonication on System A activity

The data of Fig. 3 illustrate a summary of several experiments demonstrating collectively that sonication of the reconstituted proteoliposomes for approx. 20 s increases the measurable System A activity by 50–60%. The consistency of replicate determinations within an experiment is also substantially improved by sonication. On the other hand, it should be recognised that the precise conditions of sonication are difficult to reproduce between preparations of proteoliposomes and, hence, probably contribute to the variability seen from one proteoliposome preparation to the next. As a result of this and other variables, whenever possible, experiments are performed with only one batch of proteoliposomes and comparisons of absolute velocities between experiments are avoided.

Time-course of Na^+-dependent 2-aminoisobutyric acid uptake by proteoliposomes

The time-course of 2-aminoisobutyric acid transport was measured in reconstituted proteoliposomes using the optimized conditions for the freeze-thaw cholate-dilution procedure as described above. It is apparent from the data pre-

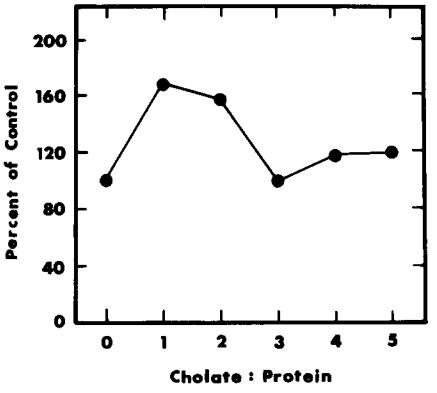

Fig. 2. Titration of the cholate to protein ratio for reconstitution of System A activity from rat liver. Solubilized membrane protein was subjected to reconstitution at cholate to protein ratios between 1 : 1 and 5 : 1. Each of the other variables was set at the optima outlined in Methods and Materials. The data are expressed as the percent of the Na^+-dependent 2-aminoisobutyric acid (AIB) (200 μM) transport activity recovered in reconstituted proteoliposomes prepared in the absence of added cholate which was 272 ± 72 pmol AIB · (mg protein)$^{-1}$ · min^{-1}. The data are the averages of four individual determinations.

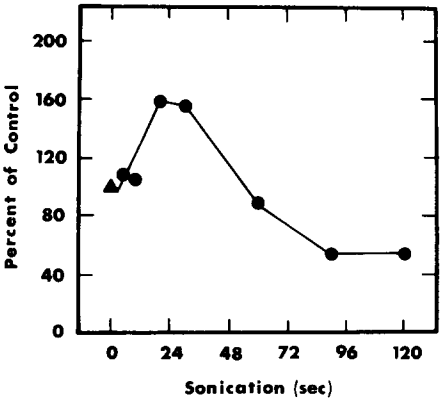

Fig. 3. Effect of sonication on rat liver System A activity in reconstituted proteoliposomes. Solubilized membrane proteins were reconstituted as described in the text using lipid to protein and cholate to protein ratios of 20 : 1 and 1 : 1, respectively. Following the dilution step the length of sonication was varied from 0 to 120 s. System A activity was assayed as Na^+-dependent uptake of 200 μM 2-aminoisobutyric acid (AIB) and the results are expressed as the percent of the velocity obtained without sonication which was 323 ± 20 pmol AIB · (mg protein)$^{-1}$ · min^{-1} (triangle).

sented in Fig. 4 that the Na^+-dependent uptake of 2-aminoisobutyric acid is linear for at least 10 min. This time-course indicates that the absolute velocities measured are similar to those seen with freshly isolated vesicles, but the length of time during which Na^+-dependent transport is detected is considerably different [11]. In the latter case, the Na^+-dependent 2-aminoisobutyric acid uptake reaches a maximum velocity after 1 min or less and exhibits a rapid overshoot, presumably the result of dissipation of the Na^+ gradient. The data of Fig. 4 demonstrate that the artificially-imposed Na^+ gradient is maintained for a longer period of time with proteoliposomes than with freshly isolated vesicles and thus, the time-scale over which Na^+-dependent transport is observed is expanded correspondingly.

Comparison of System A reconstitution between normal and hormone-induced liver tissue

A variety of hormones are known to regulate hepatic System A activity [2]. Studies involving inhibitors of macromolecular synthesis have suggested that the glucagon-mediated stimulation of hepatic System A results from the transcriptional control of synthesis of a glycoprotein [5,8]. To show that the hormone-induced transport activity can be recovered following solubilization and reconstitution at a rate proportional to that measured in isolated vesicles, plasma membranes were prepared from rats that had been injected several hours prior to sacrifice with either glucagon alone or with the combination of glucagon and dexamethasone. Glucocorticoids such as dexamethasone are known to act synergistically with glucagon with regard to regulation of hepatic System A activity [29]. As shown in Table I, the isolated vesicles from glucagon-treated rats retained the stimulated transport activity seen in whole cells and the co-administration of dexamethasone provided an additional enhancement of nearly 2-fold above that obtained with glucagon alone. Previous studies had shown that the level of hormone-induced transport measured in fresly isolated membrane vesicles was proportional to the degree of enhancement in the intact cells from which those membranes were isolated [11]. Following solubilization and reconstitution of the membrane vesicles containing stimulated System A activity two observations become apparent. First, the hormone-induced transport is recovered in the reconstituted proteoliposomes (Table I). Second, the specific activity of System A-mediated uptake in the reconstituted proteoliposomes is proportional to that

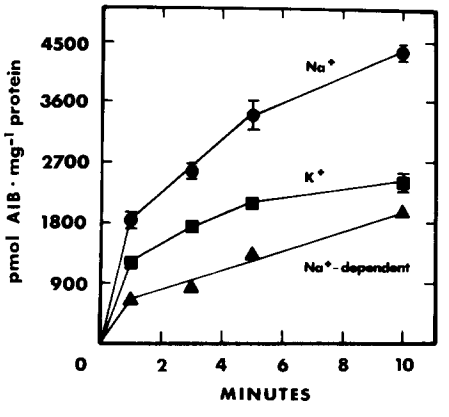

Fig. 4. Time-course of System A-mediated 2-aminoisobutyric acid (AIB) transport in reconstituted proteoliposomes. Rat liver membrane proteins were reconstituted into proteoliposomes using the optimized conditions described in the text. The Na^+-dependent uptake of 200 μM 2-aminoisobutyric acid was measured for the indicated length of time at room temperature. The data are the averages $\pm$ S.D. of at least three determinations. Where not shown, the standard deviation bars are within the symbol.

TABLE I

RECONSTITUTION OF HORMONE-INDUCED SYSTEM A ACTIVITY

Plasma membrane vesicles were isolated by the method of Prpic et al. [21] and assayed for Na^+-dependent 2-aminoisobutyric acid (AIB) uptake as described in Materials and Methods. Following solubilization of the proteins from the same membrane preparation, reconstitution of the System A activity was performed using the optimized conditions outlined in text. The results are the averages of 2–4 individual assays and were quantitatively reproducible between experiments using different membrane preparations.

Hormone treatment	Activity (pmol AIB $\cdot$(mg protein)$^{-1}\cdot$min^{-1})	
	Plasma membrane vesicles	Reconstituted proteoliposomes
None	114	197
Glucagon	514	422
Glucagon plus dexamethasone	1267	1147

present in the freshly isolated membrane vesicles.

To eliminate the possibility that the hormone-dependent stimulation of System A activity in membrane vesicles was the result of a regulatory enzymatic activity or a post-translational modification occurring within the isolated membranes, solubilized membrane protein fractions from both normal and glucagon-stimulated cells were reconstituted separately or as a mixture of equal amounts of membrane protein from each preparation. If such a regulatory factor were enzymatic or present in excess in the membranes from hormone-treated animals, the mixed proteoliposomes should exhibit a transport activity significantly greater than the average of the two preparations when assayed individually. The velocities for the normal and glucagon-induced membranes were 583 ± 135 and 1363 ± 84 pmol · (mg protein)$^{-1}$ · min^{-1}, respectively, for an average of 973. When the two solubilized protein fractions were mixed and then subjected to reconstitution, the resultant velocity was 939 ± 113 pmol · (mg protein)$^{-1}$ · min^{-1}.

Discussion

For a variety of nutrient transport systems, a wealth of information has been published concerning the characteristics and proporties at the cellular level, yet only a few of the membrane proteins that catalyze organic solute transport have been identified. Wright and his co-workers have successfully used protein-labelling techniques to identify the two proteins believed to represent the intestinal proline carrier [30] and the intestinal glucose carrier [31]. Protein modification has also been the basis for the possible identification of a Na^+-dependent bile acid transporter in liver tissue [32,33]. At the molecular level, one of the transport proteins that has been characterized extensively is the Na^+-independent glucose carrier [34]; the gene for this transporter has been cloned recently [35] and immunological and recombinant approaches have yielded structural information about the protein molecule itself [36]. Regardless of how one identifies and/or purifies a carrier protein, in the final analysis a functional assay for transport of the respective nutrient molecule must be devised. In general, this has been achieved through reconstitution of the transport protein into artificial liposomes [37].

The System A activity present in freshly isolated rat liver plasma membrane vesicles has been shown to be proportional to that present in hepatocytes from which the vesicles were derived [11]. The data presented here illustrate further that the hepatic System A activity that is induced by glucagon is tightly associated with the plasma membrane of the cell, but can be solubilized with either ionic (cholate) or non-ionic ($C_{12}E_9$) detergents and then recovered by reconstitution in proteoliposomes. Specifically, our results demonstrate three important points with regard to reconstitution of the stimulated hepatic System A activity. (1) Hepatic System A activity can be easily and reproducibly solubilized and reconstituted, although the conditions used in the present protocol do not indicate any significant degree of selectivity during this process for the carrier versus the other membrane proteins. (2) The glucagon-stimulated portion of System A-mediated transport is recovered in the reconstituted proteoliposomes and does not appear to be the result of a regulatory or enzymatic function present in the membranes from the glucagon-treated rats. Membranes from glucagon-treated animals do not cause an elevation of transport activity when mixed with membranes containing basal System A activity. (3) The hormone-induced System A transport activity present in the isolated membrane vesicles is retained completely in the corresponding proteoliposomes prepared from them. In addition to the data presented in Table I, the latter point has been underscored by comparison of uptake rates in freshly isolated vesicles and the resulting proteoliposomes from a large number of rat liver plasma membrane preparations.

As mentioned above, System A activity has been reconstituted from mouse fibroblasts [13] and Ehrlich ascites tumor cells [14–16]. Using a protocol similar to the one described in this report, our laboratory has been successful also in preparing proteoliposomes containing System A activity solubilized from Ehrlich cells [28]. Both the Ehrlich cell and rat liver plasma membrane represent excellent sources of this transport activity and therefore, attempts at purification of the carrier protein from either of these membranes

become feasible. In addition, the development of a reconstitution assay for the activity from rat liver in particular will allow further investigation of the extensive hormonal [1,2] and adaptational [38,39] regulation associated with the System A carrier in this tissue.

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References

1 Kilberg, M.S. (1982) J. Membrane Biol. 69, 1–12
2 Kilberg, M.S., Barber, E.F. and Handlogten, M.E. (1985) Curr. Topics Cell. Regul. 25, 133–163
3 Freychet, P. and LeCam, A. (1978) Proc. CIBA Found. 55, 247–268
4 Kilberg, M.S., Han, H.-P., Barber, E.F. and Chiles, T.C. (1985) J. Cell. Physiol. 122, 290–298
5 Handlogten, M.E., Barber, E.F., Bracy, D.S. and Kilberg, M.S. (1986) Mol. Cell. Endocrinol. 43, 61–69
6 Kilberg, M.S. (1986) Trends Biochem. Sci. 11, 183–186
7 Pariza, M.W., Butcher, F.R., Kletzien, R.F., Becker, J.E. and Potter, V.R. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4511–4515
8 Barber, E.F., Handlogten, M.E. and Kilberg, M.S. (1983) J. Biol. Chem. 258, 11851–11855
9 Quinlan, D.C., Todderud, C.G., Kelley, D.S. and Kletzien, R.F. (1982) Biochem. J. 208, 685–693
10 Rosenthal, N.R., Jacob, R. and Barrett, E. (1985) Am. J. Physiol. 248, E581–587
11 Schenerman, M.A. and Kilberg, M.S. (1986) Biochim. Biophys. Acta 856, 428–436
12 Samson, M. and Fehlmann, M. (1982) Biochim. Biophys. Acta 687, 35–41
13 Nishino, H., Tillotson, L.G., Scheller, R.M., Inui, K.-I. and Isselbacher, K.J. (1978) Proc. Natl. Acad. Sci. USA 75, 3856–3858
14 Cecchini, G., Payne, G.S. and Oxender, D.L. (1978) Membrane Biochem. 1, 269–278
15 Im, W.B. and Spector, A.A. (1980) J. Biol. Chem. 255, 764–770
16 McCormick, J.I., Silvius, J.R. and Johnstone, R.M. (1985) J. Biol. Chem. 260, 10614–10622
17 Handlogten, M.E. and Kilberg, M.S. (1984) J. Biol. Chem. 259, 3519–3525
18 Kasahara, M. and Hinkle, P.C. (1977) J. Biol. Chem. 252, 7384–7390
19 Karlish, S.J.D. and Pick, U. (1981) J. Physiol. 312, 505–529
20 Alfonso, M., Kandrach, M.A. and Racker, E. (1981) J. Bioenerg. Biomembranes 13, 375–391
21 Prpic, V., Green, K.C., Blackmore, P.F. and Exton, J.H. (1984) J. Biol. Chem. 259, 1382–1385
22 Zak, B. and Cohen, J. (1961) Clin. Chim. Acta 6, 665–670
23 Markwell, M.A.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978) Anal. Biochem. 87, 206–213
24 Bray, G.A. (1960) Anal. Biochem. 1, 279–285
25 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477–5487
26 Pick, U. (1981) Arch. Biochem. Biophys. 212, 186–194
27 Ochoa, E.L.M., Dalziel, A.W. and McNamee, M.G. (1983) Biochim. Biophys. Acta 727, 151–162
28 Schenerman, M.A., Racker, E. and Kilberg, M.S. (1985) J. Cell Biol. 101, 189a
29 Kletzien, R.F., Pariza, M.W., Becker, J.E. and Potter, V.R. (1975) Nature 256, 46–47
30 Wright, E.M. and Peerce, B.E. (1985) J. Biol. Chem. 259, 14893–14996
31 Peerce, B.E. and Wright, E.M. (1984) Proc. Natl. Acad. Sci. USA 81, 2223–2226
32 VonDippe, P., Drain, P. and Levy, D. (1983) J. Biol. Chem. 258, 27–68
33 VonDippe, P. and Levy, D. (1983) J. Biol. Chem. 258, 8896–8901
34 Wheeler, T.J. and Hinkle, P.C. (1982) in Membranes and Transport (Martonosi, A.N., ed.), Vol. 2, pp. 161–167, Plenum Press, New York
35 Mueckler, M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, W.J., Lienhard, G.E. and Lodish, H.F. (1985) Science 229, 941–945
36 Mueckler, M. and Lodish, H.F. (1986) Cell 44, 629–637
37 Racker, E. (1985) Reconstitutions of Transporters, Receptors, and Pathological States, Academic Press, Orlando, FL
38 Guidotti, G.G., Borghetti, A.F. and Gazzola, G.C. (1978) Biochim. Biophys. Acta 515, 329–366
39 Shotwell, M.A., Kilberg, M.S. and Oxender, D.L. (1983) Biochim. Biophys. Acta 737, 267–284