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Proalbumin Is Processed to Serum Albumin in COS-1 Cells Transfected with cDNA for Rat Albumin

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Synthesis and processing of rat albumin were investigated in COS-1 cells transiently expressing rat albumin. Analysis using isoelectric focusing revealed that serum-type albumin, which is indistinguishable from the counterpart isolated from rat hepatocyte cuture medium, was secreted from the transfected COS-1 cells, indicating that proalbumin is effectively converted into serum albumin in the COS-1 cells, if not completely. Furthermore methylamine was found to cause the diminution of serum albumin released from the cells, substantiating that the proteolytical conversion of proalbumin occurs in the Golgi complex before discharge from the COS-1 cells.

The biosynthetic precursor (proalbumin) of serum albumin has a basic hexapeptide extension, which flanked with the N-terminus of authentic serum albumin through two arginine residues (1). The conversion of proalbumin to serum albumin is believed to occur at the Golgi cisternae and/or secretory vesicles before discharge from the hepatocyte (2-5). These findings raise the possibility that the proteolytic processing of proalbumin is catalyzed by a convertase which is akin to the enzymes involved in the proteolytic processing of high molecular mass precursors of peptide hormones and neuropeptides (6).

Abbreviation used: SDS, sodium dodecyl sulfate.

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Most recently a calcium-dependent protease activity in a Golgi fraction from rat livers was suggested to be involved in the conversion of proalbumin to serum albumin (7, 8). In our study proalbumin purified from cultured hepatocytes, which had been pulse-labeled with [^{35}S]methionine, was used as a substrate (8). However, since proalbumin is rapidly converted to serum albumin in the hepatocyte, it is difficult to obtain radioactive proalbumin in quantity enough to carry out many assays through several chromatographic procedures.

In the present study we have transfected COS-1 cells with cDNA for rat albumin to obtain radioactive proalbumin in a large amount, since only precursor forms of insulin (9), opiomelanocortin (10), cholecystokinin (11) were found to be secreted from COS cell lines transfected with the respective cDNAs. Contrary to our expectation, proalbumin was found to be effectively processed to serum albumin in COS-1 cells.

MATERIALS AND METHODS

Cells COS-1 cells were maintained in culture in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and were grown at 37°C in an atomosphere of 5% CO2, 95 % ambient air.

Plasmid and Transfection pSV2ALB is a expression vector encoding a full length of rat albumin and its construction will be published somewhere. 10 ug of pSV2ALB was transfected to 5 x 10^6 COS-1 cells using a electroporation apparatus. The transfected cells were inoculated in a 10-cm culture dish and cultured for 48 h before use.

Labeling of Cells and Immunoprecipitation Transfected cells or non-transfected cells were preincubated in the absence or presence of 5 mM methylamine in methionine-free MEM for 30 min and labeled with 100 μCi of [^{35}S]methionine in fresh methionine-free MEM for 2 h in the absence or presence of methylamine. After the medium was removed, the cells were lysed in phosphate buffered saline containing 1% Triton X-100, 0.5% sodium deoxycholate and 0.05 % sodium dodecyl sulfate (SDS). Both the cell lysates and media were centrifuged at 15, 000g for 10 min and the supernatants were used for immunoprecipitation as described previously (12, 13). [^{35}S]Methionine-labeled proalbumin and serum albumin were prepared from hepatocyte cell lysate and culture medium, respectively, as described previously (13, 14).

Polyacrylamide gel electrophoresis Polyacrylamide gel electrophoresis in the presence of SDS was carried out on 10% gel according to Laemmli (15). Isoelectric gel electrofocusing was done as described previously (14). After electrophoresis, gels were fixed and processed for fluorography as described previously (13).

Immunocytochemistry COS-1 cells transiently expressing rat albumin were fixed and processed for immunocytochemistry using anti-rat serum albumin IgG Fab' fragment conjugated with peroxidase as described previously (16) and observed under a light microscope.

RESULTS and DISCUSSION

An attempt to express rat albumin in COS-1 cells was first made by MaCracken and Fishman (17), however, a secreted albumin was found to migrate slightly ahead of the authentic rat serum albumin on SDS-polyacrylamide gel, suggesting that the expressed albumin may be truncated.

In our study albumin was found to be a major secretory protein released from COS-1 cells transfected with the cDNA for rat albumin (Fig. 1, lane 6) and indistinguishable from the counterpart of hepatic origin on SDS-polycarylamide gels (Fig. 1, lane, 1). Acidotropic amine (methylamine), which is known

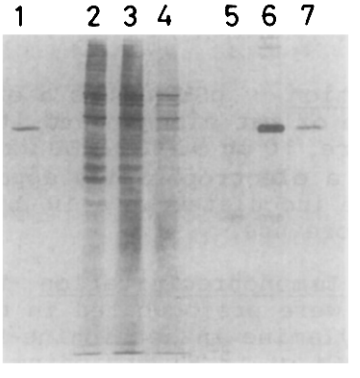
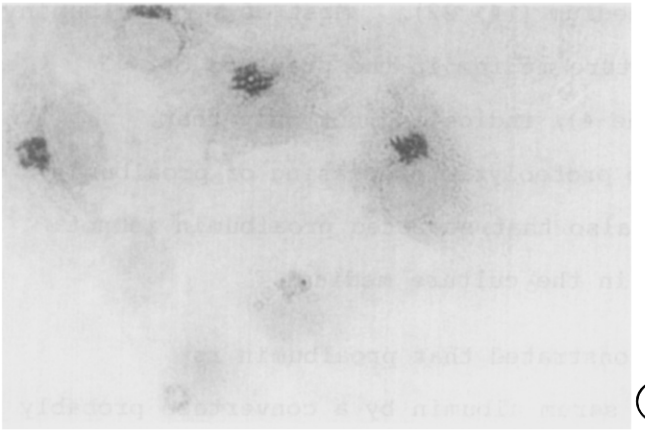


Figure 1. Expression of rat albumin in COS-1 cells. COS-1 cells, not transfected or transfected with cDNA for rat albumin, were cultured for 48 h before use. The cells were labeled with 100 μCi of [^{35}S]methionine in the absence (lanes 2, 3, 5 and 6) or presence (lanes 4 and 7) of 5 mM methylamine. After 2 h of incubation, media (lanes 5-7) were removed and cell lysates (lanes 2-4) were prepared. Aliquots of each medium and cell lysate were analyzed by SDS-polyacrylamide gel electrophoresis. Lane 1, [^{35}S]methionine-labeled rat serum albumin from hepatocyte culture medium. Lanes 2 and 5, non-transfected COS-1 cells.

to accumulate in the acidic trans Golgi region (18, 19), inhibited the secretion of albumin from the transfected cells (Fig. 1, lane 7) as in the cases of hepatocytes (14) and HepG2 cells (20).

Furthermore albumin was found to be markedly concentrated at the juxtanuclear position (Fig. 2), indicating that albumin is highly concentrated at the Golgi in the transfected COS-1 cells as demonstrated in primary cultured rat hepatocytes (16).

Fig. 3 shows an isoelectric focusing pattern of albumin synthesized and secreted from the transfected COS-1 cells. Rat proalbumin, which has three extra arginine residues in its prosequence (1), is well separable from the authentic serum albumin by virtue of their different pIs (12, 14, 21). We found two bands in the culture medium of the transfected COS-1



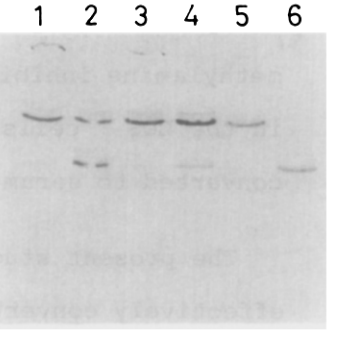


Figure 2. Immunocytochemical observation of COS-1 cells transfected with the rat albumin cDNA. COS-1 cells, transfected with the rat albumin cDNA, were cultured for 48 h before use. The cells were fixed and processed for the immunoperoxidase method. A juxtanuclear region (Golgi zone) is intensely stained. (Magnification x 1,200)

Figure 3. Isoelectric gel focusing of albumin synthesized by COS-1 cells transfected with the rat albumin cDNA. The transfected cells were labeled in the absence or presence of methylamine as described in the legend to Fig. 1. Albumin was immunoprecipitated from cell lysates (lanes 1, 3) and media (lanes 2, 4) and subjected to isoelectric gel focusing. Lanes 1 and 2, non-treated cells; lanes 3 and 4, methylamine-treated cells; lanes 5 and 6, [^{35}S]proalbumin and [^{35}S]serum albumin, respectively, from cultured rat hepatocytes.
An arrow indicates the top of gel.

cells (lane 2). One of them comigrated with a major form in the cell lysate (lane 1) and proalbumin purified from rat hepatocytes (lane 5), indicating that this band is identical to proalbumin. The other band, which has a pI value lower than that of proalbumin, migrated at the same position as the authentic serum albumin purified from hepatocyte culture medium (lane 6). Thus a significant amount of the newly synthesized proalbumin undergoes proteolytic processing and is secreted as serum albumin in the transfected COS-1 cells. In order to exclude the possibility that proalbumin is converted to serum albumin in the medium after discharge, we examined the effect of methylamine on the processing of proalbumin. In primary cultured rat hepatocytes acidotropic amines cause marked swelling of the Golgi complex and concomitantly inhibits the proteolytic processing of proalbumin, resulting in the release of proalbumin into the medium (14, 22). Most of serum albumin disappeared from the culture medium in the presence of methylamine (Fig. 3, lane 4), indicating not only that methylamine inhibits the proteolytic processing of proalbumin in the COS-1 cells, but also that secreted proalbumin is not converted to serum-type in the culture medium.

The present study demonstrated that proalbumin is effectively converted to serum albumin by a convertase probably located at the acidic <u>trans</u> Golgi region in COS-1 cells. Considering that proforms of insulin, opiomelanocortin and cholecystokinin, which have concensus amino acid motifs with pairs of basic amino acid residues at the cleavage sites are reported not to be processed in COS-1 or COS-7 cells (9-11), it is conceivable that a protease involved in the cleavage of proalbumin may be different from those involved in the processing of the above high molecular mass precursors.

However, proalbumin is not only a protein which becomes processed in the COS cells, since proforms of somatostatin (23) and von Willebrand factor (24) were reported to be converted to mature forms in the COS cells. In addition to representing an effecient expression system in conjunction with SV40-derived vector, COS cell lines provide a model system for processing of protein precursors which have only one cleavage site (23,24 and this study), but not multiple sites (9-11).

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