Biosynthesis and Packaging of Carboxypeptidase D into Nascent Secretory Vesicles in Pituitary Cell Lines

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Metallocarboxypeptidase D (CPD) is a membrane-bound trans-Golgi network (TGN) protein. In AtT-20 cells, CPD is initially produced as a 170-kDa endoglycosidase H-sensitive glycoprotein. Within 30 min of chase, the CPD increases to 180 kDa and is resistant to endoglycosidase H as a result of carbohdrate maturation. CPD also undergoes an activation step required for binding to a substrate affinity resin. Blocking the protein exit from the endoplasmic reticulum inhibits the increase in molecular mass but not the step required for affinity column binding, suggesting that enzyme activation precedes carboxypeptidase maturation and that these reactions occur in distinct intracellular compartments. Only the higher molecular weight mature CPD enters nascent secretory vesicles, which bud from the TGN of permeabilized AtT-20 and GH₃ cells. The budding efficiency of CPD into vesicles is 2–3-fold lower than that of endogenous proopiomelanocortin in AtT-20 cells or prolactin in GH₃ cells. In contrast, the packaging of a truncated form of CPD, which lacks the cytoplasmic tail and transmembrane domain, was similar to that of proopiomelanocortin. Taken together, the results support the proposal that CPD functions in the TGN in the processing of proteins that transit the secretory pathway and that the C-terminal region plays a major role in TGN retention.

Many bioactive peptides are synthesized from larger proteins by proteolytic processing that occurs in different organelles. Several endoproteases, which cleave at basic amino acid containing sites, have been described: prohormone convertase-1 (PC1)α (also known as PC3), PC2, PC4, PACE4, PC5α and -B (also known as PC6A and -B), and PC7 (also known as PC8 and LPC) (1–6). Following the endoprotease step, a carboxypeptidase is usually required to remove residual Lys and/or Arg residues from the C terminus of the peptide (7, 8). In some cases, further modification results in a C-terminal amide residue; this reaction is catalyzed by the enzyme peptidylglycine α-amidating monoxygenase (9).

Until recently, carboxypeptidase E (CPE, also known as carboxypeptidase H, enkephalin convertase, and E.C. 3.4.17.10) was thought to be the only carboxypeptidase involved in the generation of peptide hormones and neurotransmitters (7, 8). However, studies on Cpefat/Cpefat mice have suggested that another carboxypeptidase contributes to peptide processing in addition to CPE (10). The Cpefat/Cpefat mice have a point mutation in the coding region of the CPE gene, which inactivates the enzyme resulting in its degradation prior to secretion (10, 11). These mice have reduced levels of several peptide hormones and neurotransmitters and elevated levels of proteolytic-processing intermediates containing C-terminal basic residues (10, 12, 13); this finding is strong evidence that CPE plays a major role in peptide processing. However, fully processed peptides are present in moderate levels in Cpefat/Cpefat mice, raising the possibility that another carboxypeptidase contributes to peptide processing.

Carboxypeptidase D (CPD) was discovered during a recent search for enzymes with carboxypeptidase-like enzymatic properties (14). Both CPE and CPD have substantial activity at pH values in the 5–6 range (14), which is consistent with the internal pH of the intracellular compartments where processing is thought to occur (15, 16). However, the intracellular localization of CPE and CPD differ; CPE is predominantly in the secretory vesicles of the regulated pathway, whereas CPD is enriched in the TGN and immature secretory granules (17, 18). The physical properties of the two enzymes are also different. CPE is a 50–56-kDa protein that is present both as a soluble and peripheral membrane-associated form, whereas CPD is a type I 180-kDa transmembrane glycoprotein (14). Recently, rat (19) and human (20) CPD cDNAs have been sequenced and were found to be homologs of duck gp180, a protein that binds duck hepatitis B virus particles (21). Duck gp180 and rat and human CPD contain three repeats of a 50-kDa CPD-like domain, followed by a transmembrane domain and a 56 residue cytosolic tail (19–21). Several other enzymes in the secretory pathway are also membrane-bound proteins, such as furin, PC5B, and peptidylglycine α-amidating monoxygenase (22–24). These membrane-bound proteins are located in the TGN, although they also cycle to the cell surface (25, 26).

The purpose of the present study was to examine the biosynthesis and sorting of endogenous CPD into nascent secretory vesicles in the AtT-20 and GH₃ cell lines. These cells express CPD mRNA (19) and have been extensively used to study the biosynthesis and intracellular trafficking of peptide hormones and processing enzymes. The results of the pulse-chase analysis in the present study are consistent with the previous finding that CPD is present in the TGN and cycles from the cell surface back to the TGN (17) in AtT-20 cells. In addition, the finding that newly synthesized CPD is able to enter nascent secretory vesicles that bud from the TGN, albeit at a reduced efficiency relative to endogenous prohormones and hormones, is further evidence that CPD functions in the TGN and immature secre-
tory vesicles in the processing of peptides and proteins. Our results also demonstrate that the cytoplasmic tail and/or transmembrane domain of CPD play an essential role in retaining the enzyme in the TGN.

MATERIALS AND METHODS

Pulse-Chase Analysis—Wild type AtT-20 cells in 60-mm plates were incubated for 1 h in Dulbecco’s modified Eagle’s medium lacking methionine and then radiolabeled (pulsed) with [35S]Met for 15 min, washed 3 times with media, and chased for various times at 37 °C. In some experiments, the chase was performed in media containing brefeldin A (5 μg/ml), cycloheximide (100 μg/ml), or at 15 or 20 °C. Following the chase, the media were removed, and the cells were washed once with phosphate-buffered saline. Cells were then frozen on dry ice after adding 0.5 ml of 10 mM NaAc buffer at pH 5.5.

For the analysis of the active form of CPD, the cells were thawed and sonicated, and the buffer was adjusted to 1% Triton X-100 and 1 mM NaCl in 50 mM NaAc, pH 5.5. The homogenate was centrifuged at 30,000 g for 30 min, and the supernatant was subjected to purification on a 0.5-ml p-aminobenzoylarginine-Sephrose 6B affinity column as described (14, 27). Cell medium was also analyzed on the affinity resin after first adjusting the pH to 5.5 with 50 mM NaAc. CPD was eluted from the affinity column using 2 ml of Tris buffer, pH 8, containing 50 mM NaAc, 0.5 mM phenylmethylsulfonyl fluoride, and 5 mM Arg, and 0.01% Triton X-100. Aliquots of the affinity column eluate were analyzed on a 8% denaturing polyacrylamide gel, which was then treated with Fluoro-hance and exposed to x-ray film (Kodak). Quantitation of autoradiograms was performed using an image analysis system as described (28). All autoradiograms used for quantitation were in the linear range of the system.

For the isolation of CPD and CPE by immunoprecipitation, aliquots of the extracts described above were used. In some experiments, cells were extracted directly with 2% SDS using the standard immunoprecipitation protocol (29). Immunoprecipitation was performed using polyclonal antisera against purified rat CPD, purified duck CPD (gp180), the C-terminal 57 residues of duck CPD, or the C-terminal 9 residues of rat CPE (11, 27). Antisera-bound CPD was isolated using protein A-Sepharose 4B pellet in polyacrylamide gel-loading buffer containing 1% SDS for 5 min, and the immunoprecipitated material was analyzed on denaturing 6% polyacrylamide gels as described above. In some experiments, the immunoprecipitated material was incubated in the presence of N-glycosidase F (New England Biolabs), endoglycosidase H (New England Biolabs), or neuraminidase from Vibrio cholerae (Roche Molecular Biochemicals) for 16 h at 37 °C. Prior to incubation with the protease-free glycosidases, the samples were boiled to inactivate any contaminating proteases. Following digestion, the samples were analyzed on denaturing polyacrylamide gels as described above.

Permeabilized Cell Preparation and in Vitro Incubations—The preparation of permeabilized cells by a swell-release method was described previously (30, 31). Basically, cells were radiolabeled for 10 min with [35S]Met, chased for 120 min (or shorter times) at 30 °C, and incubated in cold hypotonic buffer (15 mM KCl, 10 mM HEPES, pH 7.2) for 5 min. After swelling, the cells were scraped off the plate with a rubber policeman and resuspended in 90 mM KCl, 10 mM HEPES, pH 7.2, and 1 mM MgCl2, as described (30, 31). Cells used for these analyses included wild type GH3 cells, AtT-20 cells expressing gp180 (18), and AtT-20 cells expressing gp180ΔC-term, a truncated form of duck CPD that lacks the cytosolic tail and transmembrane region.2 The incubation conditions for these experiments consisted of approximately 5 × 106 permeabilized cells, 20 mM HEPES, pH 7.3, 125 mM KCl, 2.5 mM MgCl2, 1 mM ATP, 200 μM GTP, 10 mM creatinine phosphate, 160 μg/ml creatinine phosphokinase (ATP-regenerating system), 0.5 mM phenylmethylsulfonyl fluoride, and 5 μg/ml Trasylol. Incubation for 90 min at 37 °C under these conditions is sufficient to reconstitute both prohormone processing and nascent secretory vesicle release from the TGN (30, 31).

The assay for nascent secretory vesicle budding was based on quantitating the release of radiolabeled hormones into a 15,000 × g supernatant following an in vitro incubation (31, 33). Following the incubation, permeabilized cells were pelleted and lysed in detergent, and the lysates and supernatants containing nascent secretory vesicles were immunoprecipitated with antisera to either rat CPD, duck CPD (gp180), CPE, prolactin, or ACTH (a gift of Dr. Richard Mains, Johns Hopkins University) (this antiserum also recognizes propiomelanocortin and intermediate processing forms of ACTH) as described (31). Immunoreactive material was resolved by SDS-polyacrylamide gel electrophoresis and detected by fluorography. Band intensities were quantitated using a Molecular Dynamics model 300A computing densitometer and Image Quant 3.3 software (Molecular Dynamics, Sunnyvale, CA).

RESULTS

The biosynthesis of CPD was investigated in AtT-20 cells using metabolic labeling with [35S]Met and two techniques to isolate the radiolabeled CPD, substrate affinity chromatography and immunoprecipitation. After a 15-min pulse with radioactive CPD followed by purification of the CPD on a substrate affinity column, a single 170-kDa form of CPD was detected (Fig. 1). During the 30 min chase period, the apparent molecular mass of the radiolabeled CPD increased to 180 kDa, and the amount of radiolabeled CPD recovered from the affinity column also increased severalfold (Fig. 1). Quantitation of the results from four separate experiments showed a reproducible 3-fold increase in the amount of affinity-purified radiolabeled CPD upon early chase times (Fig. 2). Following this increase, the level of radiolabeled CPD remained constant for several hours and then decreased (Figs. 1 and 2). After 20 h, the cellular level of radiolabeled CPD was approximately 30% of the peak value. No radiolabeled CPD was detected in the media for any of the time points investigated (Fig. 1).

To compare the relative amount of CPE and CPD produced by the same cells, the affinity columns were first eluted with high pH buffer (to elute CPE) before eluting the CPD with Arg. Analysis of the CPE-containing fractions showed a major band of approximately 55 kDa (not shown). The intensity of this band was substantially greater than that of the radiolabeled CPD. Because the affinity columns appear to bind both proteins quantitatively, the AtT-20 cells synthesize approximately 50–

\[ \text{FIG. 1. Pulse-chase analysis of CPD in AtT-20 cells. Cells were pulsed for 15 min, chased for the indicated period of time, and extracted as described under “Materials and Methods.” Equal proportions of cell extracts and media were subjected to purification on a p-aminobenzoylarginine-Sephrose 6B affinity column, and the column eluates were fractionated on a denaturing 8% polyacrylamide gel. The positions of prestained size standards (Bio-Rad) are indicated.} \]
100-fold more CPE than CPD, after adjustment for the number of Met residues in each protein. Quantitation of the radiolabeled CPE from four separate experiments showed a 35% decrease over the first hour followed by a slower decrease over the remainder of the chase period (Fig. 2). As previously reported (34), radiolabeled CPE was detected in the media after 1 h of chase and accumulated with increasing chase time (not shown).

When the radiolabeled CPD in the pulse-chase extracts was isolated by immunoprecipitation, its apparent molecular mass increased from 170 to 180 kDa during the 1-h chase period, but no increase in the amount of radiolabeled protein was evident (Fig. 3). Similar results were obtained when using antisemur raised against either the mature form of rat CPD (Fig. 3) or against its C-terminal region (data not shown). No differences in gel mobility were detected in comparing the extraction procedures used for the affinity column procedure (Triton X-100 plus NaCl) with the procedure used for immunoprecipitation (SDS) (data not shown). Thus, the increase in the amount of radiolabeled CPD recovered from the affinity column with time presumably represents an activation step, possibly protein folding, that is required for binding to the substrate affinity resin. In contrast to the increase in radiolabeled CPD seen during short chase periods with the affinity purification method, the decrease in CPD levels upon long chase periods was comparable for both the affinity purification and immunoprecipitation, indicating that the reduction is because of loss of CPD protein and not because of less efficient binding to the affinity column (not shown).

To investigate whether changes in glycosylation contributed to the increase in apparent molecular mass of CPD, affinity purified material was treated with glycosidases. N-Glycosidase F, which removes all N-linked sugars, reduced the size of the protein isolated immediately after the pulse (i.e. the 170-kDa form) to 147 kDa and the form after chase (i.e. the 180-kDa form) to 150 kDa (Fig. 3). Neuraminidase digestion, which removes terminal sialic acid, decreased the apparent molecular mass of the 180-kDa species to 147 kDa but had no effect on the 180-kDa form (Fig. 3). Thus, the 170-kDa species is endoglycosidase H sensitive, although the 180-kDa form is endoglycosidase H resistant, suggesting that the smaller form is predominantly localized to the ER or cis-Golgi, whereas the larger species is present in the medial or trans-Golgi.

To further study the biosynthesis of CPD, the pulse-chase was performed under several conditions that trap proteins in the ER followed by isolation using a substrate affinity resin (Fig. 4). To test whether the increase in the amount of CPD recovered on the affinity resin was because of increased synthesis of CPD, cycloheximide was used. When cycloheximide was added to the chase medium to block further protein synthesis, the amount and size of CPD was identical to that isolated from control untreated cells (Figs. 4 and 5, X). Brefeldin A added during the chase prevented the increase in apparent molecular mass but not the amount of CPD recovered from the affinity resin (Figs. 4 and 5, B). Similarly, when the chase was performed at 15 °C, the increase in size was prevented but not the amount of radiolabeled CPD bound to the affinity resin (Figs. 4 and 5, I). Incubation at 20 °C, which prevents the exit of vesicles from the trans-Golgi network (30, 35), partially blocked the size increase (Figs. 4 and 5, 20 °C). Taken together, these results suggest that the activation step is an ER event because treatments that block the exit from the ER do not prevent the activation.

To determine if CPD is packaged into nascent secretory vesicles, a permeabilized cell assay was used. This involves pulse-labeling the cells with [35S]Met followed by a 2-h chase at 20 °C to trap the radiolabeled proteins in the TGN, followed by permeabilization of the cells and analysis of the proteins present in nascent secretory vesicles, which are released from the TGN during subsequent in vitro incubation at 37 °C (31). For these experiments, A1A-1T cells were used as well as the rat anterior pituitary GH3 cell line because preliminary data determined these cells have relatively high levels of CPD. In addition, permeabilized GH3 cells have been used extensively to investigate vesicle budding from the TGN (36) and vesicle fusion with the plasma membrane (37). In GH3 cells, newly synthesized CPD was approximately 170 kDa (Fig. 4B, lane 1). When the cells were chased for 2 h at 20 °C, the majority of the protein remained as the 170-kDa form, although a small
amount was present as a 180-kDa protein (Fig. 6A, lane 2). Only the larger form of CPD entered nascent secretory vesicles as found for the CPD in GH3 cells, the budding assay was performed on cells that had been chased for only 30 or 60 min at 20 °C prior to generating a permeabilized cell preparation. For these experiments, cells expressing duck CPD (gp180) were used to improve the signal in the budding assay. After 60 min of chase at 20 °C, both 170- and 180-kDa forms of duck CPD were detected (Fig. 6E, lanes 5–8), which is consistent with the results with endogenous CPD in AtT-20 cells (Figs. 1, 3, and 4) and endogenous CPD in GH3 cells (Fig. 6A). The mature 180-kDa form of duck CPD in AtT-20 cells entered the nascent vesicles more efficiently than the immature 170-kDa form (Fig. 6E, lane 8). When the cells were chased for 30 min at 20 °C prior to permeabilization and vesicle budding, the 170-kDa form of CPD was predominant (Fig. 6E, lanes 1–4). Although only small amounts of the 180-kDa form were detected at this early chase time, the relative amount of this form in the nascent vesicles was greater than the 170-kDa form. Very low levels of the 170-kDa form of duck CPD were detected in budded vesicles after 30 min of chase at 20 °C (Fig. 6E, lane 4). It is possible that the vesicles that contain the 170-kDa form at the early chase times were derived in part from the ER or early Golgi compartments.

We hypothesized that the inefficient budding of CPD was a consequence of its retention in the TGN mediated by its C-terminal region. To test this idea, permeabilized AtT-20 cells were prepared from a cell line expressing gp180ΔC-term, a truncated form of CPD, which lacks the cytoplasmic tail and membrane anchor (Fig. 7). Significantly, the budding of vesicles containing gp180ΔC-term was generally similar to that of POMC and CPE (Table I), suggesting that the C-terminal region (cytoplasmic tail and/or transmembrane domain) of CPD functions in part as a retention sequence to decrease the efficiency of CPD packaging into nascent vesicles.

**DISCUSSION**

A major finding of the present study is that the turnover of CPE and CPD are substantially different in AtT-20 cells. This difference presumably reflects the intracellular location of each enzyme. CPD is an integral membrane protein that is predominantly localized to the TGN (17), whereas CPE is present in secretory vesicles as soluble and peripheral membrane-associ-
Met and chased for 2 h at 20 °C as outlined in Fig. 6 and under "Materials and Methods." The permeabilized cells were pulse-labeled with [35S]Met and chased for 2 h at 20 °C as outlined in Fig. 6 and under "Materials and Methods." The permeabilized cells were incubated for 90 min at 37 °C in the absence (lanes 1, 2, 5, and 6) or presence (lanes 3, 4, 7, and 8) of an energy-generating system. Following incubation, nascent secretory vesicles (S) were separated from residual permeabilized cells (P) by brief centrifugation, and the pellets and supernatant fractions were immunoprecipitated with rabbit antiserum to the N-terminal region of duck CPD (gp180). The immunoprecipitated material was resolved by SDS-polyacrylamide gel electrophoresis as described under "Materials and Methods." EGS, energy-generating system.

Fig. 7. A C-terminally truncated form of duck CPD (gp180 AC-term) enters nascent secretory vesicles more efficiently than gp180. Top, schematic representation of gp180 and gp180 AC-term. gp180 AC-term contains residues 1–1307 of gp180 and lacks the transmembrane domain (TM) and cytoplasmic tail. Bottom, stable AtT-20 cell lines expressing gp180 or gp180 AC-term were pulse-labeled with [35S]Met and chased for 2 h at 20 °C as outlined in Fig. 6 and under "Materials and Methods." The permeabilized cells were incubated for 90 min at 37 °C in the absence (lanes 1, 2, 5, and 6) or presence (lanes 3, 4, 7, and 8) of an energy-generating system. Following incubation, nascent secretory vesicles (S) were separated from residual permeabilized cells (P) by brief centrifugation, and the pellets and supernatant fractions were immunoprecipitated with rabbit antiserum to the N-terminal region of duck CPD (gp180). The immunoprecipitated material was resolved by SDS-polyacrylamide gel electrophoresis as described under "Materials and Methods." EGS, energy-generating system.

The large increase in the amount of radiolabeled CPD detected during the 1 h chase period, as determined by binding to a substrate affinity resin (Fig. 1), was not observed when the CPD was isolated by immunoprecipitation (Fig. 3) indicating that CPD requires an activation step for binding to the affinity resin. Although most peptide-processing endopeptidases are initially produced as inactive precursors that require cleavage of a propeptide for activation (39), peptide-processing carboxypeptidases do not typically require proteolytic activation. For example, proCPD and CPD are both enzymatically active (40, 41). It is unlikely that CPD undergoes proteolytic activation because the N terminus of the active form of CPD corresponds to the sequence immediately downstream of the signal peptide cleavage site (19, 20). Also, the activation step does not correlate with the increase in apparent molecular weight that also occurs during the first 30–60 min of synthesis. Furthermore, brefeldin A or incubation at 15 °C blocked the increase in size but had no effect on the amount of CPD bound to the affinity resin (Fig. 4). These observations suggest that the activation step occurs in the ER. Because the activation of CPD does not substantially alter the size of the protein, it is likely that this activation reflects the folding of the CPD into a conformation that is able to bind to the substrate affinity resin.

The increase in the apparent molecular weight of CPD that occurs approximately 30 min after protein synthesis appears to result from changes in glycosylation, because the mobility of CPD on SDS-polyacrylamide gel electrophoresis is increased upon the removal of N-linked carbohydrates (Fig. 3). It is likely that the modification of CPD leading to the shift in apparent molecular mass from 170 to 180 kDa occurs in the TGN because it is blocked by either brefeldin A or incubation at 15 but not 20 °C (Figs. 4 and 6). Brefeldin A causes the collapse of the Golgi apparatus, the redistribution of Golgi proteins into the ER (42), and blocks protein transport to the TGN (43). Incubation at 15 °C also blocks the exit of proteins from the ER, whereas incubation at 20 °C blocks the exit from the TGN (30, 35). The partial effect of the 1-h chase at 20 °C (Fig. 4) on the size shift of CPD in the AtT-20 cells is presumably because of incomplete transport of this protein to the TGN at the reduced temperature; when the chase was performed for 2 h at 20 °C, the majority of the CPD was present in the larger 180-kDa form (Fig. 6). Taken together, it is likely that the increase in size of CPD largely, but not entirely, results from the addition of terminal sialic acid residues, a modification that occurs in the late Golgi and/or TGN. The possibility of other modifications (such as sulfation and palmitoylation) cannot be excluded, although studies using chlorate to block sulfation and chemical treatments to remove palmitate did not provide any evidence for these modifications of CPD (data not shown).

Interestingly, only the larger form of CPD was found to enter nascent secretory vesicles that bud from the TGN (Fig. 6). There are several possible interpretations of this observation; either the lower molecular weight form is prevented from being packaged into nascent secretory vesicles or the enzymes that mediate the size increase are enriched or activated in the budded vesicles so that the processing occurs soon after budding. The former possibility is consistent with the size increase resulting from sialylation because sialyltransferases are present in the TGN. A similar result was observed with furin; only the higher molecular weight sialylated form was found to enter nascent vesicles that bud from the TGN (38).

The efficiency of the budding of CPD is similar in the two cell lines examined and is substantially lower than that of endogenous hormones. The difference in efficiency presumably reflects the distribution of the various proteins; although CPD is enriched in the TGN, peptide hormones are primarily located in the mature secretory vesicles of neuroendocrine cells. It is possible that the cytoplasmic tail and/or transmembrane region of CPD binds to cytoskeletal proteins, thus preventing the majority of the CPD from entering nascent secretory vesicles. We speculate that a small fraction of the CPD polypeptides are modified in the tail domain (possibly by phosphorylation or dephosphorylation) allowing them to be released and packaged into the budding vesicles along with the cargo molecules. In this context, it is noteworthy that the C-terminally truncated gp180 AC-term CPD, which lacks both a cytoplasmic tail and transmembrane domain, was sorted into nascent vesicles with an efficiency similar to that of PRL or POMC (Fig. 7 and Table I). This finding suggests that the tail and/or transmembrane domain may play an important role in TGN retention as previously hypothesized from studies on the distribution of CPD C-terminal mutants in AtT-20 cells (18). Most significantly, these data demonstrate the specificity of the vesicle budding reaction and strongly suggest that the permeabilized cell system maintains significant selectivity with respect to the packaging of cargo molecules into post-Golgi vesicles, which presumably includes both regulated and constitutive pathway vesicles. If packaging of cargo proteins were nonselective or vesicle release resulted from the random fragmentation of the Golgi apparatus/TGN in vitro, then it would be expected that similar levels of CPD and PRL or POMC would be present in the vesicle fraction; our data demonstrate that this is not the case.

The results of the present study, together with previous studies (17, 18), support the proposal that CPD functions in the TGN and/or immature secretory vesicles to process proteins that transit the secretory pathway. In this regard, CPD is similar to both furin and peptidylglycine α-amidating monoxygenase. All three enzymes contain large N-terminal luminal domains, a transmembrane domain, and a short C-terminal cytoplasmic tail. Furin is primarily located in the TGN, although it also cycles to the cell surface and back to the
TGN (25, 44). The membrane form of peptidylglycine α-amidating monooxygenase is also primarily present in the TGN, but smaller soluble forms of this protein are enriched in mature secretory granules (32). The long half-life of CPD in the pulse-chase analysis, the lack of detectable processing of CPD to shorter forms, and the absence of secretion of a significant portion of the CPD from AtT-20 cells are similar to the properties of furin. In addition, both furin (38) and CPD (Fig. 6) are detected in vesicles that bud from the TGN. Taken together, it is likely that CPD functions following the action of furin or another related endopeptidase.

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