A Hydrophobic Patch in a Charged α-Helix Is Sufficient to Target Proteins to Dense Core Secretory Granules*

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Many endocrine and neuroendocrine cells contain specialized secretory organelles called dense core secretory granules. These organelles are the repository of proteins and peptides that are secreted in a regulated manner when the cell receives a physiological stimulus. The targeting of proteins to these secretory granules is crucial for the generation of certain peptide hormones, including insulin and ACTH. Although previous work has demonstrated that proteins destined to a variety of cellular locations, including secretory granules, contain targeting sequences, no single consensus sequence for secretory granule-targeting signals has emerged. We have shown previously that α-helical domains in the C-terminal tail of the prohormone convertase PC1/3 play an important role in the ability of this region of the protein to direct secretory granule targeting (Jutras, I. Seidah, N. G., and Reudelhuber, T. L. (2000) J. Biol. Chem. 275, 40337–40343). In this study, we show that a variety of α-helical domains are capable of directing a heterologous secretory protein to granules. By testing a series of synthetic α-helices, we also demonstrate that the presence of charged (either positive or negative) amino acids spatially segregated from a hydrophobic patch in the α-helices of secretory proteins likely plays a critical role in the ability of these structures to direct secretory granule sorting.

Many enzymes and peptide hormones, including renin, insulin, and adrenocorticotropic hormone (ACTH),2 are synthesized as precursors that are proteolytically activated before secretion from the cell. In most cases, this proteolytic activation occurs only after the precursors are selectively targeted to specialized organelles found in endocrine and neuroendocrine cells called dense core secretory granules (1, 2). This process depends on the efficient co-targeting of the activating protease and its substrate from the trans-Golgi network (TGN) to nascent secretory granules, where the protease processes the precursor. Because the resulting polypeptide hormones are stored within secretory granules until the cell receives a signal for their release, this mode of protein export from the cell is called the regulated secretory pathway.

Several protein domains have been implicated in the process of sorting proteins to dense core secretory granules. The function of these domains can be grossly subdivided into three groups. The first group involves formation of high molecular weight protein complexes or aggregates. Indeed, many granule-targeting proteins have the ability to multimerize or aggregate, leading in most cases to the formation of electron dense cores (reviewed in Ref. 3). Expression of a variety of granule cargo proteins, including chromogranin A (4, 5) and provasopressin, pro-oxytocin, pro-opiomelanocortin (POMC), secretogranin II, and chromogranin B (5), is sufficient to induce aggregate-containing cytoplasmic vesicles in cells that do not normally contain secretory granules, although these vesicles do not have all of the characteristics of secretory granules (5). Proinsulin, POMC, and engineered proteins containing the C-terminal tail of the prohormone convertase PC1/3 also form multimers; however, this property alone is insufficient to direct granule sorting (6, 7). Thus, aggregation or multimerization may contribute to secretory granule-targeting efficiency, although it has not been shown in many cases to be essential. The second group of granule-sorting domains may act by binding cargo proteins to granule-tethered proteins. For example, carboxypeptidase E has been proposed to interact with a number of granule cargo proteins, including POMC, proenkephalin, growth hormone, and proinsulin, and to promote their retention in secretory granules, even though some of these are not enzymatic substrates of carboxypeptidase E (8–10). In addition, paired basic amino acids (which may form semi-stable complexes with granule-resident proteases) have been reported to direct secretory granule sorting in some proteins, including prosomatostatin (11), prorenin (12), and progastrin (13). Muclin, a type I membrane protein granule-resident protein, has also been suggested to act as a granule cargo “receptor” in pancreatic cells through its binding of sulfate groups on O-linked glycosylated proteins (14). Thus, a variety of interactions with “tethers” may serve to target proteins to secretory granules. The third class of granule-targeting protein domains involves the direct association of proteins with the secretory granule membrane. Peptidylglycine α-amidating monooxygenase (15), phgrin (16), and muclin (14) are all type I membrane-spanning proteins that are targeted to dense core secretory granules. Proteins may also interact, but not span, granule membranes; for example, the membrane-binding domains of the granule-resident protein carboxypeptidase E (17, 18) and the prohormone convertases PC1/3 (7, 19) and PC2 (20) are key for their targeting to dense

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2 The abbreviations used are: ACTH, adrenocorticotropic hormone; TGN, trans-Golgi network; POMC, pro-opiomelanocortin.
core secretory granules. In each of these cases, as well as in prosomatostatin (21) and the neuronal and endocrine polypeptide VGF (22), the granule-sorting domain includes one or more short α-helical domains. Taken together, these results raise the possibility that the presence of α-helical domains plays an important role in the targeting of certain proteins to nascent secretory granules. In this study, we sought to obtain a better understanding of the mechanism by which granule-sorting α-helices function. A systematic analysis of a series of α-helices varying in either their polar or non-polar residues or their amphipathicity indicated that the combination of charged residues with a hydrophobic patch in an α-helix is sufficient for targeting proteins to secretory granules.

EXPERIMENTAL PROCEDURES

Recombinant Plasmid Construction—Naturally occurring peptide fragments to be analyzed for secretory granule sorting were derived from the mouse PC1/3 (GenBank™ accession number NM_013628) and human prorenin (accession number NM_000537) cDNAs. The numbering used to identify the protein domains is relative to the initiator methionine of both proteins. Protein fragments were tested for their ability to sort heterologous proteins to secretory granules by attachment to a fragment of mouse IgG2b (referred to as Fc; illustrated in Fig. 1C) as described previously (7). Fusion proteins were constructed by selective amplification of the corresponding fragments using PCR. Attachment of the K/L/#helix (KLLKLLLKLWLKLLKLLL) (23) to the Fc fusion protein containing PC1/3-(711–740) (see Figs. 1 and 2) or the Fc protein alone (see Fig. 3) was accomplished by insertion of a double-stranded oligonucleotide with the following forward sequence: K/L/#synthetic amphipathic α-helix (KLLKLLLKLWLKLLKLLL) (23) to the Fc fusion protein containing PC1/3-(711–740) (see Figs. 1 and 2) or the Fc protein alone (see Fig. 3) was accomplished by insertion of a double-stranded oligonucleotide with the following forward sequence: K/L/# helical domains plays an important role in the targeting of certain proteins to nascent secretory granules. In each of these cases, as well as in prosomatostatin (21) and the neuronal and endocrine polypeptide VGF (22), the granule-sorting domain includes one or more short α-helical domains. Taken together, these results raise the possibility that the presence of α-helical domains plays an important role in the targeting of certain proteins to nascent secretory granules. In this study, we sought to obtain a better understanding of the mechanism by which granule-sorting α-helices function. A systematic analysis of a series of α-helices varying in either their polar or non-polar residues or their amphipathicity indicated that the combination of charged residues with a hydrophobic patch in an α-helix is sufficient for targeting proteins to secretory granules.

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Light antifade kit (Invitrogen) and visualized using a Zeiss LSM 510 confocal microscope.

RESULTS

Exposed \(\alpha\)-Helices Direct Secretory Proteins to Granules—To compare the ability of various \(\alpha\)-helical domains to redirect a constitutively secreted protein into secretory granules, we analyzed the secretory properties of a series of pulse-labeled recombinant fusion proteins in mouse pituitary AtT-20 cells in either the absence or presence of forskolin, a secretagogue that increases intracellular cAMP, resulting in the release of secretory granules. AtT-20 cells contain dense core secretory granules in which endogenous POMC is processed into ACTH by a series of proteolytic cleavages involving PC1/3 (reviewed in Ref. 29). As we have shown previously (7), a recombinant protein expressing a single chain fragment of the mouse immunoglobulin heavy chain constant region (Fc) was secreted constitutively (i.e. not retained in granules) when expressed in these cells as evidenced by both its low abundance after a 16-h chase (most of the protein had already been secreted) and its equivalent levels of secretion in the absence (constitutive secretion; \(-F\) lane) and presence (regulated secretion; \(+F\) lane) of forskolin (Fig. 1C). By contrast, attaching the region of the PC1/3 C-terminal tail comprising amino acids 711–753 to the C terminus of the Fc fragment (Fc-PC1/3-(711–753)) led to its increased retention after the 16-h chase and increased secretion in the presence of forskolin, demonstrating that the fusion protein had been stored in the regulated secretory pathway. This portion of the PC1/3 C-terminal tail contains a region predicted to form an \(\alpha\)-helix (Fig. 1A), which is crucial for its ability to direct secretory granule sorting (7). The importance of the \(\alpha\)-helix in directing sorting was confirmed by the loss of forskolin-stimulated release of the fusion protein in which the helical domain was deleted (Fc-PC1-(711–740)) (Fig. 1). To determine whether there is some unique characteristic of \(\alpha\)-helices that are capable of sorting proteins to secretory granules, we replaced the natural helix predicted to reside in the last 13 amino acids of the PC1/3 C terminus with an entirely synthetic amphipathic \(\alpha\)-helix (23). This \(\alpha\)-helix (also referred to as Hel 13-5 (28–30); K/L+W) (Fig. 1B) has been shown to bind with high affinity to both model membranes and biomembranes (30, 31) and to reorganize Golgi-specific phospholipid micelles into nanotubular structures (32). Surprisingly, substitution of the native helical peptide with the synthetic \(\alpha\)-helix (Fc-PC1/3-(711–740)K/L+W) led to regulated secretion of the fusion protein in AtT-20 cells (Fig. 1C). To further test whether the simple exposure of \(\alpha\)-helical domains on secretory proteins could be sufficient to target proteins to secretory granules, we attached the first 31 amino acids of the human prorenin prosegment to the C terminus of the Fc fragment (Fc-human prorenin-(1–31)) (Fig. 1C). Although this region of human prorenin contains two predicted \(\alpha\)-helical domains (Fig. 1B) (33), these play no role in secretory granule sorting in the native protein context (12). However, when placed C-terminal to the Fc fragment (Fc-human prorenin-(1–31)), the prorenin prosegment had the ability to confer regulated secretion to the fusion protein (Fig. 1C). Quantification of granule-sorting efficiency and comparison with the forskolin-stimulated secretion of endogenous \(\beta\)-en-


sections (arrowheads) (34). When either the Fc protein alone or the Fc protein attached to fragment 711–740 of PC1/3 was expressed, staining was localized primarily in the Golgi apparatus and perinuclear area (Fig. 2, left panels, arrowheads), indicating inefficient sorting to granules as predicted by our biochemical studies (see above). In contrast, attachment of either fragment 711–753 of PC1/3 or the human prorenin prosegment to the Fc fragment or of the synthetic K/L+W α-helix to Fc-PC1/3-(711–740) led to a dramatic increase in fusion protein detection in cytoplasmic extensions (Fig. 2, arrowheads). This staining co-localized with ACTH-containing secretory granules (Fig. 2, right panels). Thus, the steady-state distribution of the fusion proteins as determined by confocal microscopy confirms the results obtained by kinetic secretory assays and strongly suggests that any exposed α-helical domain in a secretory protein can direct the protein to dense core secretory granules.

Biochemical Characteristics of Granule-targeting α-Helices—Although the data presented above demonstrate that the presence of an α-helix is necessary for the granule-sorting activity of the protein fragments tested, they do not indicate whether an α-helix is sufficient to constitute a granule-sorting signal. To test this possibility, we attached the K/L+W helix directly to the C terminus of the Fc protein and examined its sorting to secretory granules in stably transfected AtT-20 cells. Because the fusion proteins attached to various synthetic α-helices were secreted with variable kinetics from the transfected cells (data not shown), we could not directly compare their sorting by pulse-chase analysis. For this reason and because the cellular distribution of the fusion proteins as determined by microscopy confirmed the pulse-chase analysis (Fig. 2), we chose to test for the steady-state distribution of the helix-containing fusion proteins in transfected AtT-20 cells (Fig. 3). Analysis of the distribution of the Fc protein fused to the K/L+W helix showed an identical pattern of distribution of Fc and ACTH staining in both the Golgi apparatus (Fig. 3, asterisks) and the cytoplasmic extensions (arrowheads). Thus, it appears that the K/L+W helix is sufficient to redirect the constitutively secreted Fc protein to secretory granules. To derive an estimate of the efficiency of granule sorting with this fusion protein, the fraction of Fc-expressing cells that contained the fusion protein in the granule-containing cytoplasmic extensions was determined (Table 2). The K/L+W helix-containing fusion protein was present in the cytoplasmic extensions of 70.40 ± 3.06% of the transfected cells, suggesting a high efficiency of targeting to secretory granules.

The finding that synthetic helices are sufficient to direct secretory granule targeting in fusion proteins

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**TABLE 1**

Secretory granule-sorting efficiency of fusion proteins in transfected AtT-20 cells

| Protein                     | Sorting index (+F/F−F) | ±S.E. (n) |
|-----------------------------|------------------------|-----------|
| Fc                          | 0.94 ± 0.10 (n = 8)    |           |
| Fc-PC1/3-(711–753)          | 4.82 ± 0.55 (n = 5)    |           |
| Fc-PC1/3-(711–740)          | 1.25 ± 0.39 (n = 3)    |           |
| Fc-PC1/3-(711–740)K/L+W     | 3.24 ± 0.12 (n = 4)    |           |
| Fc-human prorenin-(1–31)    | 3.25 ± 0.16 (n = 6)    |           |

*a p < 0.01 compared with Fc.

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**FIGURE 2.** A variety of α-helix-containing domains target fusion proteins to secretory granules. Stably transfected mouse pituitary AtT-20 cells were simultaneously immunolabeled with antibody to the various Fc fusion proteins (left panels) and endogenous ACTH (middle panels). The asterisks denote the Golgi apparatus, which stained for all of the fusion proteins as well as the ACTH precursor POMC. The arrowheads denote the cytoplasmic extensions, in which ACTH-containing secretory granules were concentrated. Note that the relative staining intensity of the fusion proteins (left panels, green) in the granule-containing cytoplasmic extensions increased dramatically when either of the three helix-containing domains was attached to the fusion protein. The original magnification was ×63. hProrenin, human prorenin.
permits the analysis of the biochemical requirements for the sorting activity by introducing systematic amino acid substitutions. As shown in the helical wheel projection in Table 3, the K/L+W helix is amphipathic (23) with a predicted pI of 11.4. To test for the contribution of the positive charge of the helix, we substituted the lysines with negatively charged glutamic acids. Thus, the E/L+W helix is also predicted to be amphipathic, but has a pI of 3.3 (Table 3). Nevertheless, confocal microscopy showed that the negatively charged E/L+W helix also redirected the fusion protein to secretory granules as evidenced by its co-localization with ACTH in the cytoplasmic extensions in 63.27 ± 3.37% of the cells (Fig. 3, arrowheads; and Table 2). Interestingly, replacing the glutamic acids with aspartic acids (D/L+W), while not significantly changing the pI of the helix (Table 3), led to reduced co-localization of the fusion protein with ACTH in the cytoplasmic extensions (36.17 ± 1.57%) and a more diffuse staining throughout the cell body (Fig. 3). A comparison of the distribution of the Fc fusion proteins linked to K/L+W, E/L+W, and D/L+W revealed that only the helix containing the glutamic acids (E/L+W) co-localize with POMC in the perinuclear Golgi apparatus (Fig. 3, asterisks), raising the possibility that these proteins partition physically as early as the TGN.

To test the importance of the hydrophobic face of the helices in sorting, we progressively decreased the hydrophobicity of the amino acids on that side of the helix. Accordingly, the K/V+W and K/A+W helices gradually reduced the size and hydrophobicity of the side chains on the hydrophobic face, whereas the K/A helix also removed the bulky tryptophan hydrophobic group. Although the K/V+W helix led to a degree of co-localization with ACTH comparable with that seen with the K/L+W helix (66.86 ± 1.45% in the cytoplasmic extensions), the K/A+W helix showed more staining of the fusion protein in the Golgi apparatus than in the granule-containing extensions, with only 3.20 ± 1.60% of the expressing cells showing the fusion protein present in the cytoplasmic extensions (Fig. 3 and Table 2). Additional removal of the bulky hydrophobic tryptophan residue from the uncharged face of the helix (K/A) led to detection of the fusion protein only in the Golgi apparatus (<0.9% of the fusion protein was present
in the cytoplasmic extensions). Thus, the ability of the helix to
direct proteins to secretory granules appears to correlate with
its hydrophobicity.

To test whether amphipathicity is necessary for the sorting
activity of the helix, we replaced three leucines on the hydro-
phobic face of the amphipathic helix with three positively
charged amino acids (Table 3, Non-amphipathic, Helical wheel
projection). Confocal microscopy of transfected AtT-20 cells
revealed that this non-amphipathic helix efficiently redirected
the fusion protein to secretory granule-containing cytoplasmic
extensions, where it co-localized with ACTH in 72.05 ± 1.95%
of the cells (Fig. 3 and Table 2). To determine whether a charged
face on the helix is important for its activity in directing secre-
tory granule sorting, a helix was designed with non-polar gly-
cines replacing the charged residues while maintaining
the hydrophobic face (G/L+W) (Table 3). This G/L+W helix-con-
taining fusion protein showed no significant co-localization
with POMC or ACTH in either the Golgi apparatus or the cyto-

TABLE 2
Secretory granule-sorting efficiency of Fc fusion proteins containing
various α-helical peptides with differing biochemical properties
Stably transfected AtT-20 cells were immunostained as described in the legend to
Fig. 3, and the percentage of Fc-expressing cells with staining in granule-containing
cytoplasmic extensions was assessed in 100 separate cells in each of three independ-
ent experiments. Values are the means ± S.E.

| Fc fusion protein | Fc-expressing cells showing localization in cytoplasmic extensions |
|------------------|---------------------------------------------------------------|
| Fc               | 2.15 ± 1.57                                                   |
| K/L+W            | 70.40 ± 3.06                                                  |
| E/L+W            | 63.27 ± 3.37                                                  |
| D/L+W            | 36.17 ± 1.57                                                  |
| K/V+W            | 66.86 ± 1.45                                                  |
| K/A+W            | 3.20 ± 1.60                                                   |
| K/A              | 0.98 ± 0.98                                                   |
| Non-amphipathic  | 72.05 ± 1.95                                                  |
| G/L+W            | 1.54 ± 1.50                                                   |

*P < 0.001 compared with Fc.

TABLE 3
Biochemical characteristics of the synthetic helices tested for granule-sorting activity
Isoelectric point and helical wheel projections were determined using the EMBOSS software package (27). Secondary structure was either predicted with the NNPREDICT
(A) (24), GOR III (B) (25), and PROF (C) (26) algorithms or determined by circular dichroism (D) (23). Hydrophobic cluster analysis was carried out by the method of
Gaboriaud et al. (28). The diamonds represent the positions of the non-polar glycine residues. Enclosed sequences represent hydrophobic patches.

| Peptide name | Primary sequence | pI  | Predicted structure | Helical wheel projection | Hydrophobic cluster projection |
|--------------|------------------|-----|---------------------|--------------------------|-------------------------------|
| K/L+W        | KLLKLLLKLWLKLLKL | 11.4| helix<sup>A-D</sup> |                          |                               |
| E/L+W        | ELLEELLELWLELELL | 3.3 | helix<sup>A-C</sup> |                          |                               |
| D/L+W        | DLLDLLDLWLDDLDLL | 3.1 | helix<sup>A-C</sup> |                          |                               |
| K/V+W        | KVVKVVKVVKVVKVVV | 11.4| helix<sup>A,B</sup> strand<sup>C</sup> | |                               |
| K/A+W        | KAAKAACKAKAAKAAA | 11.4| helix<sup>A-C</sup> |                          |                               |
| K/A          | KAAKAACKAAKAAKAAA | 11.4| helix<sup>A-C</sup> |                          |                               |
| Non-amphipathic | KRLKLLLKLWLKLLKLK | 12.5| helix<sup>A-C</sup> |                          |                               |
| G/L+W        | GLLGLLLGLWLGLLGLL | 6.1 | helix<sup>A-C</sup> |                          |                               |
plasmic extensions of the transfected cells, but rather showed a weak and diffuse staining pattern in permeabilized cells (Fig. 3).

Surprisingly, when the cells were prepared under non-permeabilizing conditions, a strong generalized staining pattern for the G/L+W helix-containing fusion protein was detected, consistent with distribution of the fusion protein on the cell surface (Fig. 4, lower right panel). This staining pattern was not seen in cells expressing the fusion protein containing the very similar helix in which the glycines were replaced with lysines (Fig. 4, lower right panel). Indeed, we have been unable to detect secretion of the K/L+W peptide at its C terminus were stained with fluorescent antibody to the Fc protein as described in the legends to Figs. 2 and 3, but without permeabilization. Note that, although the G/L+W helix-containing Fc fusion protein showed little intracellular staining (see Fig. 3), abundant staining was detected in the absence of permeabilization, suggesting localization to the cell surface (upper right panel). In contrast, the K/L+W peptide did not direct the Fc fusion protein to the cell surface (upper right panel). The original magnification was ×63.

DISCUSSION

In this study, we have provided evidence that a variety of α-helical domains can direct a linked secretory protein to dense core secretory granules. Although it is clear that α-helices are present in numerous secretory proteins that do not enter secretory granules, placement of these helices in the context of our fusion proteins may expose them, making them able to interact with membranes or other granule-sorting components in the secretory pathway. This would explain the ability of the prorenin α-helices to direct granule sorting in the fusion protein, whereas they have no such ability in the native protein. The α-helices in the C-terminal tail of PC1/3 may themselves be masked until the enzyme undergoes a conformational change in the TGN, where the tail has been proposed to unfold and to render the enzyme fully active (29, 35). In this model, the autocatalysis of the C-terminal tail would have to take place after formation of the immature secretory granule because the tail would provide the anchor for retention of PC1/3 in the nascent granule buds.

The most surprising finding of this study is that a completely synthetic α-helix is sufficient to redirect a constitutively secreted protein into the regulated secretory pathway, thereby confirming that it is the helix itself, and not adjacent sequences, that directs the secretory granule sorting of the fusion protein. This finding made it possible to systematically characterize the biochemical requirements for this sorting activity. The initial peptide that we tested (K/L+W) was shown previously to form an amphipathic helix and to rearrange Golgi-enriched liposomes into nanotubular structures in vitro (23). For direct comparison of the activity of this helix in in vitro liposome deformation and in vivo secretory granule sorting, we included the bulky hydrophobic tryptophan residue placed originally in this helix to measure tryptophan quenching in vitro (23). Our results demonstrate that the presence of the hydrophobic leucine and tryptophan residues in the helix played a critical role in conferring sorting activity (Fig. 3 and Table 2). In addition, although there was no strict requirement for amphipathicity in the helix (non-amphipathic helix) (Fig. 3 and Table 3), there did seem to be a correlation between the ability of the helix to direct sorting and clustering of hydrophobic residues (Table 3) and the degree of hydrophobicity of the residues in the cluster (Leu > Val > Ala). In addition to the requirement for hydrophobic residues, sorting helices also required segregated charged residues because removal of the charges (e.g. G/L+W) completely eliminated the subcellular sorting of the fusion protein to the secretory granule and caused the fusion protein to accumulate on the cell surface. The finding that both negatively and positively charged amino acids could be substituted in the helix supports a role of the charge beyond a primary recognition event in sorting (e.g. with another protein or with a charged lipid). The seemingly less efficient sorting of the helix containing glutamic acids (E/L+W) compared with the fusion proteins containing other charged helices remains difficult to understand. Notably, the aspartic acid side chain is the smallest among the charged amino acids we tested, raising the possibility that larger charged side chains are more efficient in directing granule sorting. Interestingly, the natural sorting helices of PC1/3 (Fig. 1), PC2 (20), and carboxypeptidase E (18) are rich in glutamic acids. One possible explanation for the role of the charged amino acids is that they maintain a shallow interaction of the helix with the inner leaflet of the TGN membrane. This model is supported by the behavior of the G/L+W peptide, in which elimination of the charged residues caused the helix to behave as if it were anchored in the membrane and unable to be secreted.

The importance of the hydrophobic amino acids and the relative unimportance of the charge of the polar residues have been reported in other membrane-deforming helices. Both positively and negatively charged synthetic α-helices are equally capable of deforming liposomes into tubular structures.

FIGURE 4. The G/L+W fusion protein is localized to the cell surface. Transfected AtT-20 cells stably expressing an Fc fusion protein with either the K/L+W or G/L+W peptide at its C terminus were stained with fluorescent antibody to the Fc protein as described in the legends to Figs. 2 and 3, but without permeabilization. Note that, although the G/L+W helix-containing Fc fusion protein showed little intracellular staining (see Fig. 3), abundant staining was detected in the absence of permeabilization, suggesting localization to the cell surface (upper right panel). In contrast, the K/L+W peptide did not direct the Fc fusion protein to the cell surface (upper right panel). The original magnification was ×63.
in vitro (32). Mutation of the hydrophobic residues in a non-membrane-spanning α-helical region of the membrane-interacting protein epsin abolishes its ability to curve membranes (36). Similarly, mutation of hydrophobic residues in Sar1p (a component of the coat protein II complex) abolishes its ability to deform lipid vesicles in vitro, whereas mutation of the charged residues within the helix has no effect (37). Our results suggest that α-helices involved in sorting proteins to secretory granules might also act by interacting directly with membranes. Indeed, PC1/3 (7, 19), PC2 (20), and carboxypeptidase E (18) have been reported to bind membranes through their helical components, permitting targeting to secretory granules and subsequent secretion in the extracellular milieu.

In summary, we have shown that a variety of natural and non-natural α-helices have the capacity to redirect a constitutively secreted protein to the regulated secretory pathway. The major characteristic of these sorting α-helices is the presence of both a hydrophobic cluster and segregated charged amino acids.

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