**SUPPLEMENTARY MATERIAL**

## EXPERIMENTAL PROCEDURES

*$Ca^{2+}$ titrations monitored by fluorescence spectroscopy.* $Ca^{2+}$-binding constants were determined by monitoring the intrinsic (tryptophan) fluorescence during $Ca^{2+}$ titration in 0.15 M KCl, 2 mM Tris, pH 7.3. The protein concentration was 25 µM, and $Ca^{2+}$-free protein was titrated with increasing amounts of metal ion. The intensity at each point was corrected for dilution. $Ca^{2+}$ titrations were also performed in the presence of 120 µM ANS and 10 µM secretagogin in 1 mM DTT, 0.15 M KCl 2 mM Tris/HCl, pH 7.3. The experimental data were fitted according to

$$I = I_0 + \left(I_p - I_0\right)\frac{Y \cdot K_A}{1 + Y \cdot K_A} \qquad \text{(I)}$$

Where Y is the free $Ca^{2+}$ concentration, $K_A$ is the apparent binding constant, and $I_0$ and $I_p$ are the intensities for the free and bound state, respectively.

## RESULTS

**$Ca^{2+}$ binding to secretagogin as monitored by ANS- and Trp- fluorescence.**

Secretagogin displays an increase in Trp fluorescence upon $Ca^{2+}$ binding (Fig. 2A). The fluorescence intensity was monitored as a function of total $Ca^{2+}$ concentration, and the response is changing in the range of calcium concentrations corresponding to the low affinity sites. The $Ca^{2+}$ affinity was estimated by fitting equation 1 to the data (not shown) yielding an average affinity for the low affinity sites as $Log_{10}K = 4.5 \pm 0.3$, or $K_D = 28 \pm 14$ µM, in 0.15 M KCl, 2 mM Tris/HCl pH 7.3, based on six replicates. We also fitted an extended binding model to the data allowing for multiple macroscopic binding constants. By this procedure, the data could be fitted by one high affinity site and one or more low affinity sites. The binding constants for the low affinity sites did not change by the inclusion of a strong site. Due to the

low protein concentration used in Trp fluorescence studies (30 times lower than the $K_D$ of the weak sites) it is not possible to determine the stoichiometry of $Ca^{2+}$ binding.

$Ca^{2+}$ binding to secretagogin in the presence of ANS leads to a large increase in ANS fluorescence (Fig. 2B), and provides an indirect means of studying the conformational changes following $Ca^{2+}$-binding to the protein. Fitting equation 1 to the $Ca^{2+}$ titrations as monitored by ANS fluorescence yields $Log_{10}K = 4.3 \pm 0.3$, or $K_D = 52 \pm 26$ μM, based on six replicates in 0.15 M KCl, 2 mM Tris/HCl pH 7.3. These numbers are in fair agreement with the results from titrations monitored by Trp fluorescence. However, several of the replicates were not well fitted by equation 1, and required analysis with a model including at least two macroscopic $Ca^{2+}$-binding constants. In these cases, the best fits were obtained with $Log_{10}K_2 > Log_{10}K_1$, implying positive cooperativity of $Ca^{2+}$ binding among the low affinity sites. Although the titration monitored by Trp and ANS fluorescence does not provide the high precision obtained using the competitive chromophoric chelator method, it does confirm that the binding constants obtained by the chelator method are in the correct range.

**TABLES**

SUPPLEMENTARY MATERIAL Table 1. Analysis of peptides from limited proteolysis of secretagogin by MALDI-TOF mass spectrometry.

| $Ca^{2+}$ 5 minutes digestion | | | | | $Ca^{2+}$ 30 minutes digestion | | | |
|---|---|---|---|---|---|---|---|---|
| Measured mass | Calculated mass | Δmass (ppm) | position | | Measured mass | Calculated mass | Δmass (ppm) | position |
| 3882.000 | 3882.450 | 115.900 | 24-56 | | 3881.420 | 3882.450 | 265.300 | 24-56 |
| 4379.700 | 4379.994 | 67.100 | 12-47 | | 4379.350 | 4379.994 | 147.000 | 12-47 |
| 4446.000 | 4446.107 | 24.100 | 24-61 | | 4445.590 | 4446.107 | 116.300 | 24-61 |
| 4673.370 | 4673.414 | 9.300 | 24-63 | | 4672.200 | 4673.414 | 259.800 | 24-63 |
| 5341.000 | 5341.092 | 17.200 | 12-56 | | 5341.000 | 5341.092 | 17.200 | 12-56 |
| 5561.400 | 5562.411 | 181.700 | 30-77 | | 5561.180 | 5562.411 | 221.300 | 30-77 |
| 5905.250 | 5904.749 | -84.800 | 12-61 | | 5905.470 | 5904.749 | -122.000 | 12-61 |
| 6132.290 | 6132.056 | -38.100 | 12-63 | | 6131.600 | 6132.056 | 74.300 | 12-63 |
| 6268.200 | 6268.133 | -10.600 | 24-77 | | 6268.600 | 6268.133 | -74.400 | 24-77 |
| 7725.400 | 7726.775 | 177.900 | 12-77 | | 6558.100 | 6558.485 | 58.700 | 6-61 |
| 22813.880 | 22816.087 | 96.700 | 12-204 | | 7725.100 | 7726.775 | 216.800 | 12-77 |
| | | | | | 12869.000 | 12868.763 | -18.400 | 57-165 |
| | | | | | 16185.800 | 16183.499 | -142.100 | 64-200 |
| | | | | | 22814.000 | 22816.087 | 91.400 | 12-204 |

| EDTA 5 minutes digestion | | | | | EDTA 30 minutes digestion | | | |
|---|---|---|---|---|---|---|---|---|
| Measured mass | Calculated mass | Δmass (ppm) | position | | Measured mass | Calculated mass | Δmass (ppm) | position |
| 3582.300 | 3582.078 | -62.000 | 214-246 | | 3580.800 | 3582.078 | 356.800 | 214-246 |
| 4379.350 | 4379.994 | 147.000 | 12-47 | | 3879.600 | 3880.398 | 205.600 | 116-149 |
| 5374.930 | 5375.223 | 54.400 | 144-189 | | 4206.300 | 4206.859 | 132.800 | 214-251 |
| 5905.700 | 5904.749 | -161.000 | 12-61 | | 4379.550 | 4379.994 | 101.300 | 12-47 |
| 6290.100 | 6291.193 | 173.700 | 205-260 | | 4669.200 | 4669.318 | 25.200 | 205-246 |
| 5562.700 | 5562.411 | 104.400 | 30-77 | | 5064.510 | 5064.786 | 54.400 | 116-159 |
| 7726.700 | 7726.775 | 9.700 | 12-77 | | 5561.830 | 5562.411 | 104.400 | 30-77 |
| 9838.140 | 9840.329 | 222.400 | 190-276 | | 5907.270 | 5904.749 | -426.700 | 12-61 |
| 22814.800 | 22816.087 | 56.300 | 12-204 | | 9435.830 | 9439.843 | 425.200 | 80-159 |
| | | | | | 10040.700 | 10037.518 | -316.900 | 75-159 |
| | | | | | 11388.940 | 11390.022 | 95.000 | 160-257 |
| | | | | | 12866.000 | 12868.763 | 214.700 | 57-165 |
| | | | | | 12866.000 | 12871.749 | 446.800 | 136-246 |
| | | | | | 13397.100 | 13402.377 | 393.800 | 132-246 |
| | | | | | 22809.900 | 22802.046 | -344.300 | 48-244 |
| | | | | | 22809.900 | 22816.087 | 271.200 | 12-204 |

## FIGURES

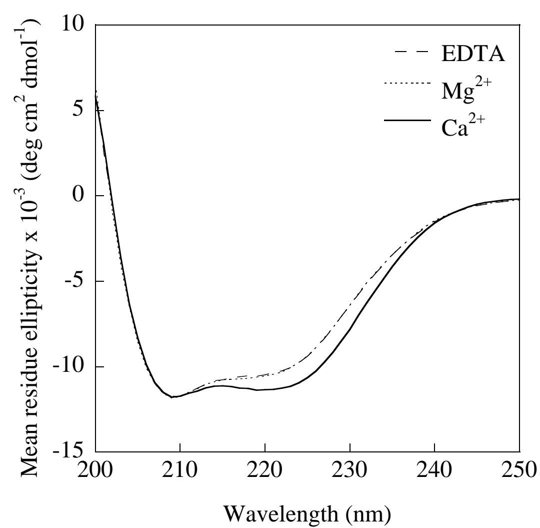
SUPPLEMENTARY MATERIAL FIG 1. **Far UV Circular dichroism spectra of secretagogin.** The far UV CD-spectrum (200-250 nm) at room temperature was recorded for a solution of 6 uM secretagogin in 1 mM DTT, 0.5 mM EDTA, 2 mM TRIS, pH 7.5 (apo-secretagogin; dashed line). 5 mM $MgCl_2$ was then added (dotted line) and finally 5 mM $CaCl_2$ was then added to the same solution (solid line).

SUPPLEMENTARY MATERIAL FIG 2. **Tryptic digestion of secretagogin**. Human recombinant secretagogin was digested with trypsin as described in the materials and methods section. Fragments were generated in the presence of 1 mM $CaCl_2$ or in the presence of 1mM EDTA and separated by SDS poly-acrylamide (16.5 %) gel electrophoresis in a Tris/Tricine buffer system.

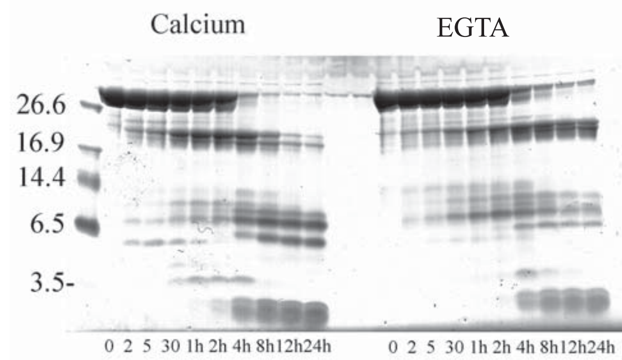
SUPPLEMENTARY MATERIAL FIG 3. **Mass spectrometric identification of protein fragments.** Tryptic fragments of secretagogin generated in 1 mM EDTA or 1 mM $CaCl_2$ at various time-points were identified by MALDI-TOF mass spectrometry. The experimentally

measured peptide masses were compared with the theoretical peptides calculated from the human secretagogin sequence by the FindPept tool (http://us.expasy.org/tools/findpept.html). Spectra generated after 5 and 30 minutes of digestion are shown. Peptides that could be assigned to a specific mass within secretagogin are indicated by the first and last residue number. Identified peptides that are similar in both forms are shown within brackets.

Supplementary material, Fig 1



Supplementary material, Fig 2



Supplementary material, Fig 3