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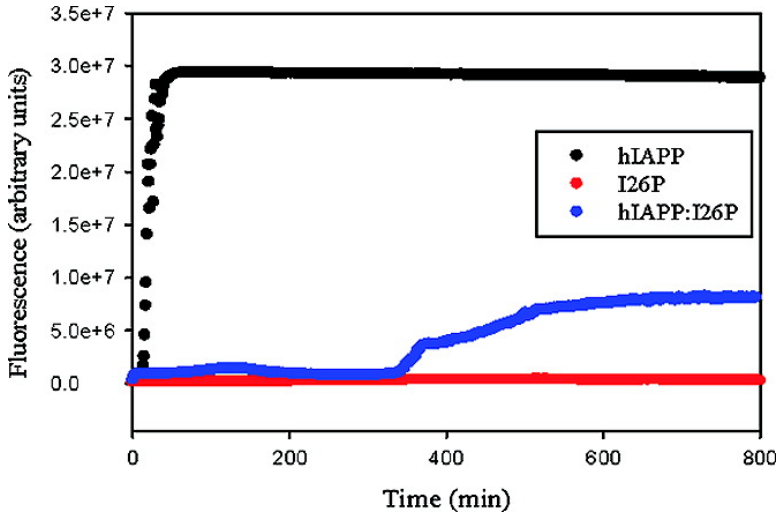


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# A Single-Point Mutation Converts the Highly Amyloidogenic Human Islet Amyloid Polypeptide into a Potent Fibrillization Inhibitor

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Protein aggregation and amyloid formation play critical roles in a range of human diseases including Alzheimer's disease, type 2 diabetes, and Parkinson's disease.[1] A common feature of type 2 diabetes is the formation of islet amyloid. Amyloid deposition in the pancreas is believed to contribute to islet cell dysfunction and to the loss of $\beta$-cell mass in type 2 diabetes.[2] Islet amyloid polypeptide (IAPP or amylin) is responsible for amyloid formation in type 2 diabetes. The peptide is synthesized in the pancreatic $\beta$-cells and is cosecreted with insulin.[3]

There is considerable interest in developing inhibitors of amyloid formation, both because of their potential therapeutic use and because they can be important reagents for mechanistic studies of amyloid fibril formation.[4] A popular approach involves the use of small peptide fragments derived from the target protein. The fragments are used as recognition motifs and variants can sometimes act as inhibitors if substitutions are incorporated or appended that block $\beta$-sheet propagation.[4] Many of the inhibitors reported to date are effective only at large molar excess, limiting their usefulness as mechanistic probes and as lead compounds for therapeutic applications. In vitro, IAPP is one of the most amyloidogenic polypeptides known and is even more prone to form amyloid than the A$\beta$ peptide. Thus, the development of effective inhibitors of wild-type IAPP amyloid formation is extremely challenging. Here we demonstrate that a single point mutant converts IAPP from a highly amyloidogenic polypeptide into a potent inhibitor of wild-type IAPP amyloid formation.

Wild-type human IAPP (hIAPP) is 37 residues in length, has an amidated C-terminus, a free N-terminus, and contains a disulfide bridge between residues 2 and 7. The sequence of hIAPP is

KCNTATCATQRLANFLVHSSNNFGA**I**LSSTNVGSNTY

Inspired by studies of multiply methylated analogs of IAPP and by proline scanning studies of a ten-residue fragment derived from the highly amyloidogenic 20 to 29 region of hIAPP,[4g,5] we examined the effects of a single proline mutant at position 26 in the full-length polypeptide, (indicated in bold in the sequence). This substitution abolished amyloid formation in the 20 to 29 fragment.[5a] Peptide-based inhibitors have been designed using the 20 to 29 fragment as the basic unit[4f] and multiple proline substitutions within this region are believed to be at least partially responsible for the lack of amyloid formation by rat IAPP.[5b,6] hIAPP and the I26P mutant of hIAPP (I26P) were synthesized and the fibrillization rate of each peptide was determined using standard fluorescence detected thioflavin-T binding studies. The quantum yield of the dye increases dramatically when bound to the amyloid cross-$\beta$ structure. The rate of amyloid fibril formation is strongly dependent upon experimental conditions and is very sensitive to sample agitation; being much faster for stirred versus quiescent samples. We conducted our assays with constant stirring since this dramatically enhances the rate of wild-type IAPP fibrillization and provides more challenging conditions for the testing of inhibitors. The I26P point mutant had a profound effect on the ability of hIAPP to form amyloid (Figure 1). The I26P point mutant was also a potent inhibitor of wild-type fibrillization, increasing the lag time by almost a factor of 20. The lag time for a 1:1 mixture of I26P and wild-type hIAPP was 292 $\pm$ 62 min (mean $\pm$ standard deviation of 6 measurements) while the lag time for wild-type hIAPP was 14 to 16 min under our conditions. The significant increase in the lag time is noteworthy since some small peptide and non-peptide inhibitors have been observed to have an effect upon the final fluorescence intensity but not upon the lag phase.[4,7] The final fluorescence intensity of the 1:1 mixture was also significantly, reduced relative to wild-type hIAPP, being a factor of 3 lower. The wild-type sample and the 1:1 mixture contained the same amount of wild-type hIAPP thus the reduction in fluorescence is not due to a lower concentration of wild-type. The effects are larger than have been observed for some fibrillization inhibitors even though many of those were examined at large molar excess.

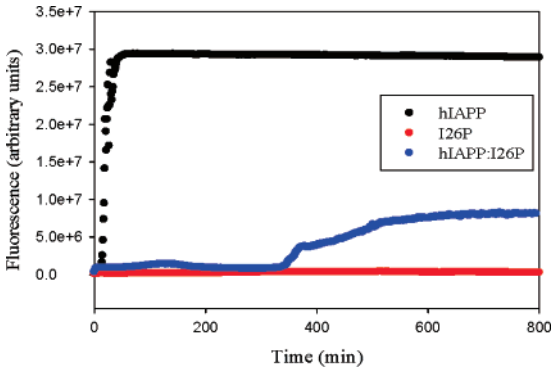


***Figure 1.*** Kinetic measurements demonstrate that the I26P point mutant is a potent inhibitor of amyloid fibril formation. Time course of amyloid fibril formation monitored by fluorescence detected thioflavin-T binding: wild-type hIAPP (black), I26P (red), and a 1:1 molar ratio mixture of wild-type hIAPP and I26P (blue). All samples contained 2% HFIP at pH 7.4, 25 °C. Samples were continually stirred throughout the experiments.

The final thioflavin-T fluorescence intensity reflects the amount of fibrillar material formed, the binding constant for thioflavin-T, and the quantum yield of the bound dye.[8] Changes in thioflavin-T fluorescence intensity between different samples are often assumed to be directly related to the amount of amyloid formed. However, changes in the binding constant or the quantum yield of the dye can contribute to any observed differences. These considerations do not alter the important observation that the lag phase of the 1:1 mixture is considerably lengthened relative to the wild-type; however, they do mean that the final fluorescence intensity should not necessarily be interpreted only in terms of the amount of amyloid formed. Thus, it is important to confirm the results of the thioflavin-T experiments using independent methods. Transmission electron microscopy (TEM) studies conducted as a function of time confirm that the single proline mutant significantly affects fibril formation (Figure 2 and Supporting Information) as do circular


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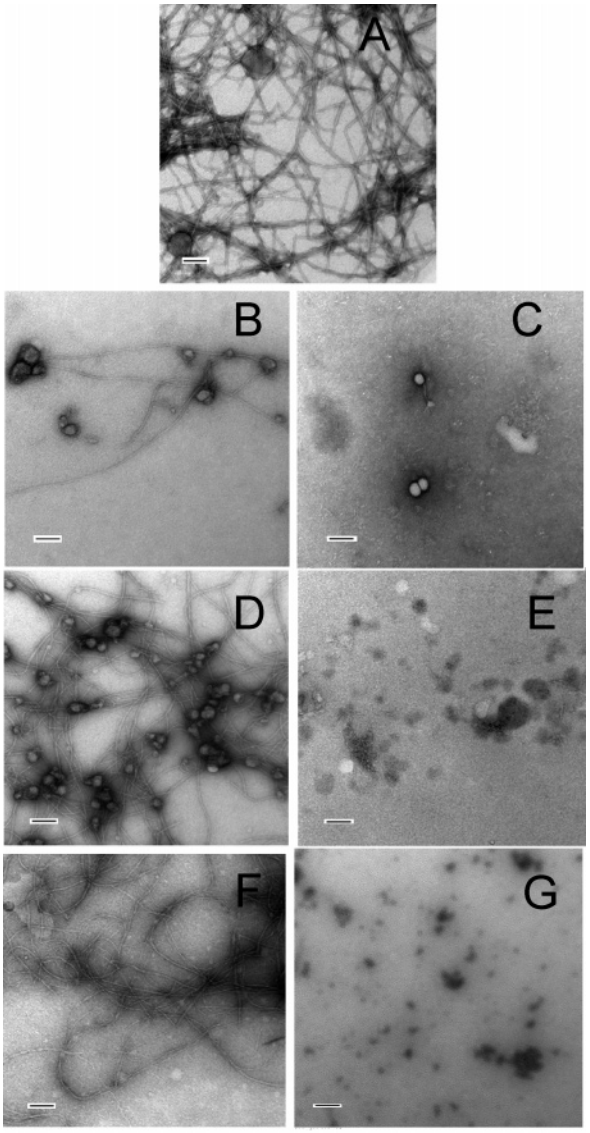


***Figure 2.*** Time dependent TEM studies confirm that the I26P point mutant is a potent inhibitor of amyloid fibril formation. (A) TEM image of wild-type hIAPP collected during the plateau region of the reaction. TEM images (C, E, G) displayed in right column are of I26P, and images (B, D, F) in the left column are of the 1:1 mixture of wild-type hIAPP and I26P. (B and C) Images collected at the midpoint of the lag phase of the 1:1 mixture; (D and E) images collected at a time corresponding to the start of the growth phase of the 1:1 mixture; (F and G) images collected at a time corresponding to the plateau region of the 1:1 mixture (600 min). Additional TEM data are provided in the Supporting Information. All samples were examined at pH 7.4. Scale bars represent 100 nm.

dichroism (CD) measurements at the end of the reactions (Supporting Information).

TEM images of wild-type hIAPP reveal dense mats of fibrils with the classic amyloid morphology (Figure 2A). In contrast, images of I26P and the 1:1 mixture collected at a time corresponding to the midpoint of the lag phase of the 1:1 sample show much less aggregated material. Few fibrils are observed in the 1:1 sample but small granular/spherical aggregates are detected (Figure 2B). Images collected at a time corresponding to the growth phase of the 1:1 mixture show that more spherilites are present and thin fibrils have developed, but the amount of fibrils is less than for the wild-type sample (Figure 2D). These TEM images are similar to ones reported for other inhibitors of IAPP.[5,7] The I26P sample shows no signs of fibril formation (Figure 2E). Images collected of the 1:1 mixture after 600 min show that the spherilites are now largely absent and thin fibril-like species have developed (Figure 2F). The I26P mutant still has not formed fibrils after 600 min (Figure 2G).

It is natural to inquire why the I26P mutant is more effective than inhibitors based on small peptide fragments. Inhibitors need to bind to growing fibrils or to prefibril species to exert their effects and a point mutant of full length hIAPP should bind more tightly than a small fragment which contains the same substitution. The increased affinity should, in turn, lead to more potent inhibition. Recent work has suggested that intermediates in hIAPP aggregation could involve association driven by interactions in the N-terminal half to two-thirds of the molecule.[9] The I26P peptide will be able to bind to such structures since the mutation is removed from this region. Subsequent conversion to amyloid fibrils would be inhibited by the presence of proline at position 26. Along these lines, it is interesting to note that the B-chain of insulin is an inhibitor of IAPP fibrillization and recent studies have shown that residues 7 to 19 of IAPP encompass the primary interaction site with the B-chain.[10]

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**Supporting Information Available:** Experimental details, supporting CD data, and additional TEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) (a) Glenner, G. G. *New Engl. J. Med*. **1980**, *302*, 1283−1292. (b) Sipe, J. D. *Crit. Rev. Clin. Lab. Sci.* **1994**, *31*, 325−354. (c) Selkoe, D. J. *Nature Cell. Bio.* **2004**, *6*, 1054−1061. (d) Cohen, F. E.; Kelly, J. W. *Nature* **2003**, *426*, 905−909.

(2) (a) Kahn, S. E.; Andrikopoulos, S.; Verchere, C. B. *Diabetes* **1999**, *48*, 241−246. (b) Hull, R. L.; Westermark, G. T.; Westermark, P.; Kahn, S. E. *J. Clin. Endo. Met.* **2004**, *89*, 3629−3643. (c) Konarkowska, B.; Aitken, J. A.; Kistler, J.; Zhang, S.; Cooper, G. J. S. *FEBS J*. **2006**, *273*, 3614−3624.

(3) (a) Westermark, P.; Wernstedt, C.; Wilander, E.; Hayden, D. W.; O'Brien, T. D.; Johnson, K. H. *Proc. Nat. Acad. Sci. U.S.A.* **1987**, *84*, 3881−3885. (b) Cooper, G. J. S.; Willis, A. C.; Clark, A.; Turner, R. C.; Sim, R. B.; Reid, K. B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8628−8632.

(4) (a) Sciarretta, K. L.; Gordon, D. J.; Meredith, S. C. *Methods Enzymol.* **2006**, *413*, 273−312. (b) Estrada, L. D.; Soto, C. *Curr. Topic. Med. Chem.* **2007**, *7*, 115−126. (c) Soto, C.; Sigurdsson, E. M.; Morelli, L.; Kumar, R. A.; Castano, E. M.; Frangione, B. *Nat. Med.* **1998**, *4*, 822−826. (d) Hughes, E.; Burke, R. M.; Doig, A. J. *J. Biol. Chem.* **2000**, *275*, 25109−25115. (e) Gilead, S.; Gazit, E. *Angew, Chem., Int. Ed.* **2004**, *43*, 4041−4044. (f) Kapurniotu, A.; Schmauder, A.; Tenidis, K. *J. Mol. Biol.* **2002**, *315*, 339−350. (g) Yan, L.; Tatarek-Nossol, M.; Velkova, A.; Kazantzis, A.; Kapurniotu, A. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* 2046−2051.

(5) (a) Moriarty, D. F.; Raleigh, D. P. *Biochemistry* **1999**, *38*, 1811−1818. (b) Westermark, P.; Engstrom, U.; Johnson, K. H.; Westermark, G. T.; Betsholtz, C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5036−5040.

(6) (a) Green, J.; Goldsbury, C.; Mini, T.; Sunderji, S.; Frey, P.; Kistler, J.; Cooper, G.; Aebi, U. *J. Mol. Biol.* **2003**, *326*, 1147−1156. (b) Abedini, A.; Raleigh, D. P. *J. Mol. Biol.* **2006**, *355*, 1066−1077.

(7) (a) Binder, W. H.; Smrzka, O. W. *Angew. Chem., Int. Ed.* **2006**, *45*, 7324−7328. (b) Cohen, T.; Frydman-Marom, A.; Rechter, M.; Gazit, E. *Biochemistry* **2006**, *45*, 4727−4735. (c) Aitken, J. F.; Loomes, K. M.; Konarkowska, B.; Cooper, G. J. S. *Biochem. J.* **2003**, *374*, 779−784.

(8) Levine, H. *Methods Enzymol.* **1999**, *309*, 274−284.

(9) (a) Knight, J. D.; Hebda, J. A.; Miranker, A. D. *Biochemistry* **2006**, *45*, 9496−9508. (b) Jayasinghe, S. A.; Langen, R. *Biochim. Biophys. Acta* 2007, published online, doi:10.1016/j bbamem 2007 01 022. (c) Abedini, A.; Tracz, S. M.; Cho, J.-H.; Raleigh, D. P. *Biochemistry* **2006**, *45*, 9228−9237.

(10) Gilead, S.; Wolfenson, H.; Gazit, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 6476−6480.

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