Identifying Structural Features of Fibrillar Islet Amyloid Polypeptide Using Site-directed Spin Labeling*

Received for publication, June 18, 2004, and in revised form, August 2, 2004 Published, JBC Papers in Press, September 8, 2004, DOI 10.1074/jbc.M406853200

Sajith A. Jayasinghe and Ralf Langen‡
From the Department of Biochemistry and Molecular Biology, Zilkha Neurogenetic Institute, University of Southern California, Los Angeles, California 90033

Pancreatic amyloid deposits, composed primarily of the 37-residue islet amyloid polypeptide (IAPP), are a characteristic feature found in more than 90% of patients with type II diabetes. Although IAPP amyloid deposits are associated with areas of pancreatic islet β-cell dysfunction and depletion and are thought to play a role in disease, their structure is unknown. We used electron paramagnetic resonance spectroscopy to analyze eight spin-labeled derivatives of IAPP in an effort to determine structural features of the peptide. In solution, all eight derivatives gave rise to electron paramagnetic resonance spectra with sharp lines indicative of rapid motion on the sub-nanosecond time scale. These spectra are consistent with a rapidly tumbling and highly dynamic peptide. In contrast, spectra for the fibrillar form exhibit reduced mobility and the presence of strong intermolecular spin-spin interactions. The latter implies that the peptide subunits are ordered and that the same residues from neighboring peptides are in close proximity to one another. Our data are consistent with a parallel arrangement of IAPP peptides within the amyloid fibril. Analysis of spin label mobility indicates a high degree of order throughout the peptide, although the N-terminal region is slightly less ordered. Possible similarities with respect to the domain organization and parallelism of Alzheimer’s amyloid β peptide fibrils are discussed.

Pancreatic amyloid deposits have been identified as a hallmark of non-insulin-dependent (type II) diabetes mellitus. More than 90% of patients with type II diabetes exhibit amyloid deposits in areas of β-cell dysfunction and death (1, 2). The major component of these deposits is the 37-residue islet amyloid polypeptide (IAPP) (3, 4), and multiple lines of investigation have provided experimental evidence implicating human IAPP amyloid deposits in type II diabetes (5–8).

Although pancreatic amyloid deposits in a diabetic patient were first identified more than a century ago, structural features of these deposits were not described until recently. Electron microscopy (EM) studies of IAPP deposits revealed the presence of long fibrillar structures (9–11). Fourier transform infrared and circular dichroism (CD) analysis of IAPP fibrils made from full-length as well as shorter fragments indicates the presence of significant amounts of β-sheet structure in the fibrillar form (12, 13). X-ray and electron diffraction studies using aligned fibrils have shown that the peptide chains are arranged in a cross-β-conformation with the individual β-strands perpendicular to the fibril axis (14, 15). Beyond these observations, little is known about the molecular details of IAPP in the fibrillar form.

Amyloid deposits have been identified in a number of human diseases, such as Alzheimer’s disease, Parkinson’s disease, and familial amyloidotic polynuropathy (16). Although the primary structure of amyloid forming proteins varies widely, most amyloid deposits appear to share common characteristics, such as the cross-β-structure, and the ability to bind thioflavin T (ThT) and Congo red (17). Because of the non-crystalline and insoluble nature of amyloid deposits, it has been difficult to obtain a detailed molecular structure of amyloidogenic proteins in the fibrillar form using conventional biophysical techniques such as x-ray crystallography and solution-state nuclear magnetic resonance (NMR) spectroscopy. In contrast, other magnetic resonance techniques, such as solid-state NMR (18–20) and electron paramagnetic resonance (EPR) spectroscopy together with site-directed spin labeling (SDSL) (21–24) have been used successfully to obtain structural and dynamic features of amyloidogenic proteins in their fibrillar form (for review, see Ref. 25).

In the present study, we used EPR spectroscopy in combination with SDSL to obtain structural features of full-length IAPP. In SDSL, a cysteine-specific nitroxide spin label is introduced at selected sites of the protein molecule, resulting in a nitroxide-labeled side chain (R1, see Fig. 1). EPR spectra of these R1 reporter groups reflect the local environment of the label and can be used to classify a given site as a loop region, as an exposed or buried site, or as a tertiary contact site (26–28). Furthermore, the distance between two R1 side chains can be estimated from EPR spectra, given the presence of spin-spin interactions between spin labels (29). We find that most regions of IAPP in its fibrillar form are arranged in a highly ordered fashion with the same residues from different strands in close proximity to one another, indicating a parallel arrangement of IAPP peptides within the fibril. The organization of IAPP in the fibrillar form is similar to that observed for Alzheimer’s Aβ peptide fibrils obtained using EPR/SDSL (23) and NMR (20) spectroscopy. These similarities suggest that the molecular architecture of IAPP and Aβ fibrils could be related.

* This work was supported by the Beckman Foundation and the Pew Scholars Program in the Biomedical Sciences (to R. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Zilkha Neurogenetic Institute, 1501 San Pablo St., Los Angeles, CA 90033. Tel.: 323-442-1323; Fax: 323-442-4404; E-mail: langen@usc.edu.

The abbreviations used are: IAPP, islet amyloid polypeptide; EM, electron microscopy; CD, circular dichroism; ThT, thioflavin T; NMR, nuclear magnetic resonance; EPR, electron paramagnetic resonance; SDSL, site-directed spin labeling; Aβ, amyloid β; HFIP, hexafluoroisopropanol; MTSL, 1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl methanethiosulfonate.

48420 This paper is available on line at http://www.jbc.org
EXPERIMENTAL PROCEDURES

Chemicals and Peptides—Hexafluorisopropanol (HFIP) and ThT were obtained from Sigma-Aldrich. Synthetic wild-type human IAPP was obtained from Bachem (King of Prussia, PA). The spin label, 1-oxyl-2-5,5-tetramethyl-35-pyrene-3-methyl methanesulfonate (MTSL), was obtained from Toronto Research Chemicals (Toronto, Canada). The thiol-reactive pyrene label, N-(1-pyrenyl)maleimide, was obtained from Molecular Probes (Eugene, OR). An analog of IAPP containing no cysteine residues (wherein the native cysteines were replaced by alanine) was synthesized using fluorenyl-9-methoxycarbonyl chemistry on an automatic peptide synthesizer (ABI 433A, Applied Biosystems, Foster City, CA). Single cysteine substituted mutants of full-length IAPP based on our cysteine-less template were purchased from Synpep Corp. (Dublin, CA). Cysteine residues were replaced by reverse phase high-pressure liquid chromatography and were characterized for correct molecular weight by electrospray mass spectrometry. Purified peptides were lyophilized and stored at −70 °C until further use. For spin labeling, purified peptides were reacted with 5-fold molar excess of MTSL for 1 h at room temperature. Unreacted MTSL was removed by reverse phase high-pressure liquid chromatography, and spin-labeled peptides were characterized for correct molecular weight using electrospray mass spectrometry.

Preparation of Peptide Stock Solutions—We prepared peptide stock solutions under conditions found to reproducibly generate unordered conformations of IAPP (30). Purified peptides were dissolved in 6 M guanidine HCl and loaded onto a spin column packed with C18 reverse phase separation media (Harvard Apparatus, Holliston, MA). After loading, the column was washed once with deionized distilled water, and the peptide was eluted using 100% HFIP. Peptide concentrations were calculated by UV absorbance at 280 nm in 6 M guanidine HCl using an extinction coefficient of 1400 M−1 cm−1 for the wild-type peptide (30) and 1160 M−1 cm−1 for the cysteine-less peptide (based on an extinction coefficient of 120 M−1 cm−1 for cysteine (31)). Stock solutions in HFIP were stored at −70 °C.

Fibril Formation—Aliquots of peptide stock solutions in HFIP were pipetted into 1.5 ml Eppendorf tubes, and the HFIP was evaporated using a speed vac system (Savant Instruments, Halbrook, NY). The dry peptide was resuspended in buffer for 1 h at room temperature. Fibril formation was monitored to proceed at room temperature for a minimum of 3 days. At the end of the incubation period, fibrils were separated from any smaller aggregates by centrifugation and repeated washing with buffer. For spin-diffusion experiments, aliquots of each R1-labeled IAPP analog in HFIP were mixed with the appropriate amount of either wild-type IAPP or cysteine-less IAPP in HFIP, and fibrils formed as described above.

EPR Spectroscopy—For EPR spectroscopy, fibril suspensions were transferred into quartz capillaries (0.6 mm inner diameter × 0.84 mm outer diameter, VitroCom, Mt. Lakes, NJ). Spectra were obtained on a Bruker EMX spectrorometer (Bruker Instruments, Billerica, MA) fitted with an ER4123D resonator. EPR spectra were obtained at room temperature using a field modulation amplitude of 1 (solution spectra) or 3 (fibrillar spectra) gauss at an incident microwave power of 12 MW with a scan range of 150 gauss. Spectra were normalized to the same number of spins using the double integral and are presented accordingly.

CD Spectroscopy—For CD spectroscopy, aliquots of peptide stock solutions in HFIP were pipetted into 1.5 ml Eppendorf tubes, mixed with 500 µl of deionized distilled water, immediately frozen in liquid nitrogen, and lyophilized overnight. The dry lyophilized peptide was dissolved in 10 mM phosphate buffer to yield 25 µM solutions prior to use and transferred into a 1-mm path-length quartz cell. Spectra of IAPP fibril suspensions in buffer were obtained from fibrils grown from freshly dissolved IAPP as described above. CD spectra were obtained using a Jasco 810 spectropolarimeter (Jasco, Inc., Easton, MD). Measurements were taken every 0.5 nm at a scan rate of 50 nm/min with an averaging time of 1 s. Typically, 20–50 scans were averaged between 190–280 nm. All spectra were corrected using appropriate backgrounds.

Fluorescence Spectroscopy—Fibril solutions for the ThT binding assay were prepared as described for CD spectroscopy. To each fibrilization reaction, 25 µM ThT was added and emission intensities were measured at 482 nm with excitation at 450 nm. Measurements were performed at room temperature with excitation and emission slit widths of 1 and 10 nm, respectively. Emission spectra of pyrene-labeled IAPP fibrils were obtained with excitation at 350 nm, a scan rate of 100 nm/min, and excitation and emission slit widths of 1 and 5 nm, respectively. For comparison, emission spectra of pyrene-labeled IAPP were normalized using the peak at 395 nm. Fluorescence measurements were carried out using a Jasco FP-6500 spectrofluorometer.

Electron Microscopy—5–10 µl of fibrillar IAPP solution was adsorbed onto glow-discharged carbon-coated copper grids. Excess sample was removed by blotting on filter paper. Grids were stained with 5 µl of 3% uranyl acetate for 3 min. After blotting excess uranyl acetate and washing with 5 µl of distilled deionized water, staining was repeated for 1 min with 5 µl uranyl acetate.

RESULTS

Native Cysteines Are Not Required for Fibril Formation by Full-length IAPP—IAPP contains two native cysteine residues that can form a disulfide bridge under oxidizing conditions (32). It is not clear, however, whether the link is always present in the fibrillar state and/or whether it is required for fibril formation in vivo. Synthetic peptides lacking the N-terminal seven residues of IAPP have been found to form amyloid fibrils with a morphology similar to that of the full-length native peptide (11), which implies that the two native cysteines are not necessary for the formation of amyloid fibrils in vitro. To determine whether fibril formation by full-length IAPP requires the two native cysteine residues, we used CD and ThT binding.

Immediately after they were dissolved in phosphate buffer, both wild-type and cysteine-less IAPP produced far UV CD spectra (Fig. 2, A and B, solid lines) with minima at ~200 nm. These spectra are characteristic of an unordered backbone structure. To observe structural changes associated with fibril formation, far UV CD spectra were collected from aged samples containing fibrillar aggregates (see EM below). For both wild-type and cysteine-less IAPP, we observed a reduction of the minimum at 200 nm, with a concomitant increase in the negative ellipticity at ~220 nm upon fibril formation (Fig. 2, A and B, dotted lines). These changes are indicative of a conformational change from an unordered to a β-sheet structure and are in agreement with published data on IAPP (12, 13).

The ability of wild-type and cysteine-less IAPP to form fibrillar structures was also monitored using ThT, a dye known to bind strongly to amyloid fibrils (33, 34). To monitor fibril formation, ThT emission intensity at 482 nm was measured at different time points (Fig. 2C). For both wild-type and cysteine-less IAPP, we observed increases in ThT emission intensity similar to those reported previously (30). In both cases, the increased occur in ThT intensity indicate the formation of fibrils with highly similar (yet not identical) kinetics. Although the subtle difference in kinetics is close to the inherent variability of the ThT assay, it is possible that the N-terminal conformation of IAPP could make minor contributions to the kinetics of fibril formation (15).

Next, we used EM to test whether the absence of the two native cysteines influenced the fibril morphology. We observed similar fibril morphologies for the wild-type and cysteine-less peptides (Fig. 2, D and E). Taken together with our CD and ThT data, these similarities imply that the absence of the two native cysteine residues does not alter the ability of full-length cysteine-less IAPP to form amyloid fibrils.

EPR Spectra of Soluble and Fibrillar Forms of Spin-labeled IAPP Analogs—To obtain insight into the molecular architecture of IAPP monomers in their soluble and fibrillar forms, we R1-labeled eight single cysteine analogs of IAPP (see “Experimental Procedures”). Cysteines were placed at selected sites.
throughout the sequence to sample structural information from different regions of the peptide. We found that R1-labeled IAPP analogs in solution gave rise to EPR spectra with sharp lines with a narrow spectral width, as illustrated in Fig. 3A (black line) for the representative R1-labeled position 21. These spectral features are characteristic of highly mobile R1 label with a rotational correlation time in the sub-nanosecond time scale in agreement with the unordered structure indicated by CD (Fig. 2A). In contrast to the soluble form, fibrillar IAPP gave rise to spectral broadening and very low signal amplitude (Fig. 3A, red line). To test for the presence of spin-spin interactions, fibrils were grown from a mixture of R1-labeled (at position 21) and unlabeled wild-type or cysteine-less IAPP. Increasing the amount of unlabeled peptide gave rise to spectra with sharper lines and reduced spectral width, confirming the presence of spin-spin interactions. These observations suggest that the two native cysteines are not required for fibril formation.

**EPR Spectra of Spin-labeled IAPP Co-mixed with Unlabeled Peptide**—To test for the presence of spin-spin interactions in the spectra obtained for fibrils from R1-labeled IAPP derivatives, we carried out spin dilution experiments wherein fibrils were grown from a mixture of R1-labeled and unlabeled IAPP. The rationale for these experiments is as follows: If unlabeled and R1-labeled IAPP co-mix in the fibril, the likelihood of an R1
Structural Features of IAPP

A mixture of R1-labeled and unlabeled IAPP (Fig. 4) showed decreased spectral amplitude and that the cysteine-less, wild-type, and R1-labeled peptides exhibited inverse line widths for sites 2R1 and 7R1 (Fig. 4, arrows). A more quantitative comparison of mobility of the R1 label can be obtained using the inverse central line width, wherein larger values indicate higher mobility. The N-terminal residues 2R1 and 7R1 exhibited inverse line widths of 0.32 and 0.27 G⁻¹, respectively, whereas sites between residues 12–29 had inverse line width values ranging from 0.13 to 0.17 G⁻¹, suggesting that the two N-terminal sites experience less immobilization than do sites within the center of the peptide.

**DISCUSSION**

Our objective was to characterize the structural organization of IAPP in its fibrillar form. Using EPR and SDSL, we found that IAPP undergoes a transition from a highly dynamic structure in solution to a very ordered, specific, and parallel structure in the fibril.

Although the mobility of all eight sites was significantly reduced in the fibrillar form, we observed variations in R1 mobility that likely indicate distinct structural regions within IAPP. We found that the central region of IAPP (residues 12R1, 21R1, 27R1, 28R1 and 29R1) exhibited the least amount of mobility. Together with our observation of the presence of pronounced spin-spin interactions within this region, this lack of mobility suggests that these residues are located within the central β-sheet-containing core of the IAPP fibril. This interpretation agrees with previous analyses of IAPP fragments showing that residues 1–7 are not required for fibril formation (15, 35, 36).

We have observed that in addition to a reduction in mobility there was the presence of spin coupling at all eight sites, suggesting that the R1 label on each site of a given peptide is in close proximity of sites from neighboring molecules (see “Discussion”).

To confirm the close proximity of R1-labeled sites using an independent approach, we employed a pyrene-labeled analog of IAPP. Fig. 3D shows the emission spectra for IAPP labeled with pyrene at the 21 position in the monomeric form in solution (black line), and after the formation of fibrillar aggregates (red line). In the case of the fibrillar sample, we observed a broad emission band at ~472 nm characteristic of excited state pyrene dimer (excimer) emission, which was not observed in monomeric IAPP. Pyrene excimers are formed from the precise stacking of two monomers that are in physical contact and proper orientation. Therefore, the occurrence of pyrene excimer fluorescence in fibrillar IAPP indicates the close proximity of pyrene molecules from neighboring IAPP peptides and is in agreement with the interpretation of our EPR data.

**EPR Spectra of Spin-diluted Fibrils**—To obtain additional details on the molecular structure of IAPP, we analyzed the mobility of R1 in the fibrillar form. Our spin dilution experiments indicate that spin-spin interactions significantly contribute to the spectral broadening and low signal amplitude observed in the fibrillar spectra. In the absence of spin-spin interactions, EPR line shapes provide information on the dynamics of R1. At sufficiently high dilution with unlabeled peptide, the EPR spectra from spin-labeled, fibrillar IAPP are determined primarily by R1 mobility. As shown in Fig. 4 (black lines), all spin-diluted spectra showed increased separation between the outer EPR lines and broad spectral lines compared with solution spectra, indicating the immobilization of R1 at all eight sites. Thus, in addition to R1-labeled sites on neighboring molecules being in close proximity to one another, each labeled site became much more ordered upon fibril formation. This immobilization was most pronounced at sites located in the central region of IAPP (sites between residues 12–29). Slightly less immobilization was observed for sites in the N terminus of the peptide as indicated by the larger signal amplitude and the slightly less narrow spectral lines for sites 2R1 and 7R1 (Fig. 4, arrows). A more quantitative comparison of mobility of the R1 label can be obtained using the inverse central line width, wherein larger values indicate higher mobility. The N-terminal residues 2R1 and 7R1 exhibited inverse line widths of 0.32 and 0.27 G⁻¹, respectively, whereas sites between residues 12–29 had inverse line width values ranging from 0.13 to 0.17 G⁻¹, suggesting that the two N-terminal sites experience less immobilization than do sites within the center of the peptide.

**DISCUSSION**

Our objective was to characterize the structural organization of IAPP in its fibrillar form. Using EPR and SDSL, we found that IAPP undergoes a transition from a highly dynamic structure in solution to a very ordered, specific, and parallel structure in the fibril.

Although the mobility of all eight sites was significantly reduced in the fibrillar form, we observed variations in R1 mobility that likely indicate distinct structural regions within IAPP. We found that the central region of IAPP (residues 12R1, 21R1, 27R1, 28R1 and 29R1) exhibited the least amount of mobility. Together with our observation of the presence of pronounced spin-spin interactions within this region, this lack of mobility suggests that these residues are located within the central β-sheet-containing core of the IAPP fibril. This interpretation agrees with previous analyses of IAPP fragments showing that residues 1–7 are not required for fibril formation (15, 35, 36).

We have observed that in addition to a reduction in mobility there was the presence of spin coupling at all eight sites, suggesting that the R1 label on each site of a given peptide is in close proximity of sites from neighboring molecules (see “Discussion”).

To confirm the close proximity of R1-labeled sites using an independent approach, we employed a pyrene-labeled analog of IAPP. Fig. 3D shows the emission spectra for IAPP labeled with pyrene at the 21 position in the monomeric form in solution (black line), and after the formation of fibrillar aggregates (red line). In the case of the fibrillar sample, we observed a broad emission band at ~472 nm characteristic of excited state pyrene dimer (excimer) emission, which was not observed in monomeric IAPP. Pyrene excimers are formed from the precise stacking of two monomers that are in physical contact and proper orientation. Therefore, the occurrence of pyrene excimer fluorescence in fibrillar IAPP indicates the close proximity of pyrene molecules from neighboring IAPP peptides and is in agreement with the interpretation of our EPR data.
Structural Features of IAPP

close proximity to the same site on neighboring molecules. This interpretation is further supported by our observation of pyrene excimer fluorescence that constrains the pyrene molecules from neighboring peptides to be within physical contact. In a recent SDSL study of tau fibrils from singly labeled tau molecules, exchange-narrowed single-line EPR spectra lacking hyperfine structure (i.e. two outer peaks) were observed (21). Such exchange narrowing requires orbital overlap of the nitroxides from many spin labels on the EPR time scale, suggesting an extended stacking of R1 labels within the fibril. We observed reduced hyperfine structure (reduced intensity of outer peaks) for sites within the proposed core region, although not as pronounced as in the case of tau; thus, we cannot exclude the presence of spin exchange and possible contact between R1 labels from neighboring molecules for these sites. It is difficult to determine the precise amount of exchange present in our spectra; nevertheless, the presence of spin exchange could be significant. To establish an approximate upper limit, we used spectral subtraction to determine the relative contribution of the hyperfine peaks. Based upon these subtractions, we found that the contribution from spin exchange could be high at all sites within the core region and up to 50–90% for positions 21, 27 and 28 (data not shown). Thus, the presence of spin coupling at all eight sites in IAPP, coupled with the observation of pyrene excimer fluorescence, indicates a parallel arrangement of strands within the fibril.

The present data on IAPP are strikingly similar to those obtained from our laboratory for the Alzheimer’s Aβ peptide (23). In the case of Aβ, we observed spin-spin interactions and EPR spectra quite comparable with those observed here. The EPR data, together with numerous solid-state NMR studies (37–39), clearly demonstrate a parallel, in-register arrangement of individual strands for Aβ. Therefore, given the similarity between our EPR data and that of Aβ, it is highly likely that a similar in-register parallel structure also applies to IAPP. In fact, a parallel arrangement might be much more common for proteins/peptides with longer core regions, such as IAPP, Aβ (23), α-synuclein (22), tau (21), and as recently suggested for the yeast prion Ure2p (40). For shorter segments and peptide fragments of Aβ (41–43) and IAPP (18, 44), however, anti-parallel β-sheet arrangements have been reported. Recently, the role of amphipathicity in determining the parallel/anti-parallel organization of amyloid fibrils has been described (45). In Aβ (1–40), the C terminus is considerably hydrophobic, whereas the charged hydrophilic residues are found toward the N-terminal half of the peptide. Gordon et al. (45) propose that this amphipathicity of Aβ (1–40) is responsible for its parallel arrangement within fibrils, whereas a lack of amphipathicity facilitates the anti-parallel arrangement of shorter fragments of Aβ. Although comparable studies have not yet been performed for IAPP, inspection of the sequence points to the possibility that amphipathicity may also play a role in the organization of IAPP within fibrils. In full-length IAPP, the N-terminal 11-residue segment is hydrophilic, containing all three charged residues of IAPP. In contrast, the remaining two-thirds of the peptide contain 10 hydrophobic residues, 6 of which are found clustered within the C-terminal 15 residues. Such a separation of hydrophobic and hydrophilic residues imparts an amphipathicity to IAPP that may favor the parallel arrangement of full-length peptides within fibrils.

The similarities between IAPP and Aβ could also extend to the organization of their respective domains, given the similarities observed between the primary sequence of IAPP and Aβ peptides (Fig. 5). O’Nuallain et al. (46) found a 65% sequence similarity when the overlap region was confined to residues 15–37, or the central core region, of Aβ. Furthermore, O’Nuallain et al. found that Aβ fibrils can act as efficient seeds for IAPP aggregation (but curiously, not the converse) (46), implying that at least under certain conditions IAPP could adopt a structure similar to that of Aβ (20, 47) in the fibrillar form. Aβ contains a central ordered region that exhibits pronounced spin-spin interactions. The N-terminal region of Aβ is of lower order overall and exhibits weaker coupling, implying that the N terminus lies outside of the core. For the two IAPP N-terminal sites, 2R1 and 7R1, we observed inverse line with values of 0.32 and 0.27 G, respectively, suggesting a lower order for these sites. Although we have not yet tested every single site in the N terminus, these two values at least are very close to those obtained for similar sites from the N terminus of Aβ (23), indicating the likelihood that the N terminus of IAPP is not within the most highly ordered core region. Furthermore, we find that the N-terminal cysteines are not required for fibril formation, and that the cysteine-less and wild-type peptides co-mix with labeled IAPP, suggesting that all three forms of the peptide can take up similar fibrillar structures. If the disulfide link between residues 2 and 7 remains intact during wild-type IAPP fibril formation in vivo, conformational constraints render it unlikely that the N-terminal region would facilitate the formation of a β-sheet structure.

In summary, our EPR/SDSL data have allowed us to identify several structural features of IAPP in the fibrillar form. Our EPR data are consistent with a situation wherein IAPP fibrils are formed from the ordered parallel arrangement of IAPP monomers. Based upon side-chain mobility, we have been able to identify distinct structural regions of IAPP fibrils. Further EPR/SDSL studies should enable us to identify additional structural features of IAPP fibrils, such as bend or turn regions (48), and ultimately to generate sufficient experimental constraints to develop a three-dimensional model of IAPP in the fibrillar form.

Acknowledgments—We thank Drs. Mario Isas and Martin Margittai for many useful discussions regarding this manuscript and thank Diana Gegala for assistance during its preparation.

![FIG. 5. Similarities between IAPP and Aβ. Some sequence similarity is observed within the ordered core regions of both peptides (vertical lines, solid for exact matches and dashed for similar residues). EPR spectral analysis indicates a similar domain distribution for both peptides (horizontal lines). The domain distribution for IAPP is based upon mobility information obtained from this study. The domain distribution of Aβ is based upon EPR and NMR data (20, 23). These similarities indicate that the structure of fibrillar IAPP may be similar to that of Aβ in the fibrillar form.](image-url)