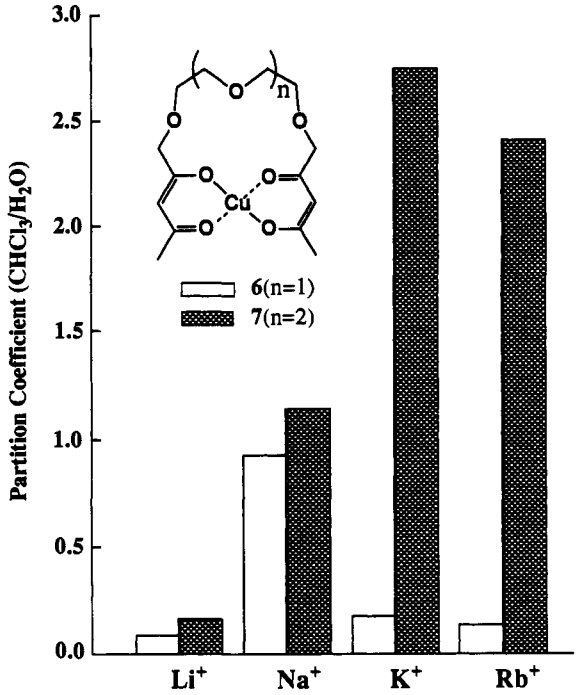

Figure 1. Partition coefficients[7] of alkali-metal picrates between $CHCl_3$ and H_2O with the use of copper-assisted coronands **6** and **7**.

structure-forming capacity of the first metal ion. This provides an interesting way of molecular organization in place of the "preorganization"[8e] which has long been a dominant principle in crown ether chemistry. The metal-assisted organization *after the chemical synthesis* may be an interesting alternative, because the ligand synthesized could be subjected further to a reversible organization to construct or destroy the structure as required. Therefore, the method is expected to lead to a new strategy for the design of supramolecular chemistry. Further studies are now actively under way.

The Structural Basis of Pancreatic Amyloid Formation: Isotope-Edited Spectroscopy in the Solid State

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The deposition of proteinaceous amyloid is characteristic of many diseases, including Alzheimer's disease (AD),[1] scrapie,[2] and type II diabetes.[3] Because amyloid is insoluble and noncrystalline, structural models of the constituent cross-β fibril are based solely on the low-resolution technique of X-ray fiber diffraction.[4] Consequently, there is little information regarding the molecular details of amyloidogenesis. We have developed a Fourier transform infrared spectroscopic (FTIR) method, based on isotropic substitution, which can discern details in amyloid structure which were previously unobservable.[5] We report herein the application of this method, which we call isotope-edited dipole coupling analysis, to a peptide amyloid related to the pancreatic amyloid of type II diabetes. An extension of the method allows the determination of the critical intermolecular interactions present in the antiparallel β-sheet structure which is the subunit of the cross-β fibril. The FTIR analysis and solid-state ^{13}C NMR studies carried out in parallel suggest that a sequence of at least four amino acids is critical in precipitating amyloidogenesis.

Table I[a]

analogue	A ^{12}C (cm^{-1})	B ^{13}C (cm^{-1})	C δ (ppm)	D line width (Hz)
S20	1628 (0)		172 (+1)	285
F23	1632 (+4)	1614 (+2)	172 (+4)	317
G24	1643 (+15)	1610 (+6)	170 (+3)	190
A25	1637 (+9)	1606 (+9)	174 (+2)	171
I26	1639 (+11)	1605 (+9)	171 (+4)	181
L27	1638 (+10)	1611 (+4)	173 (+4)	213
S28	1629 (+1)	1618 (ND)	173 (0)	322
S29	1629 (+1)		174.5 (-1.5)	386

[a] Column A lists the position of the ^{12}C amide band (± 2 cm^{-1}) and, in parentheses, the shift from the position in the unlabeled spectrum (1628 cm^{-1}). This shift reflects the total amount of dipole coupling (inter- and intramolecular) experienced by that amide. Column **B** lists the position of each ^{13}C amide I band and, in parentheses, the shift observed on isotopic dilution (5:1). The magnitude of the shift depends on the amount of intermolecular dipole coupling. For S28, the ^{13}C band was at 1618 cm^{-1} but was not observable on isotopic dilution. For S20 and S29, the ^{13}C band was not observable. Column C lists the chemical shift of each carbonyl carbon and, in parentheses, the deviation of that shift from the "unstructured" value.[13,14] For example, the chemical shift of the F carbonyl carbon in the multiconformational pentapeptide GGFGG is 176 ppm. Each value is the average of two separate experiments (deviation was <1 ppm) except for S20, which is the result of a single experiment. Column D lists the average line width of each carbonyl carbon line (two experiments; deviation ≤10%; S20 was a single experiment). Line width is related to several factors, including conformational disorder and structural rigidity.[14]

The presence of pancreatic amyloid may interfere with β cell function and cause insulin insensitivity or may be an epiphenomenon associated with type II diabetes.[3] Pancreatic amyloid comprises a 37-residue peptide known as the islet amyloid polypeptide (IAPP).[3] We synthesized[6] a 10 amino acid peptide (AcHN-SNNFGAILSS-$CONH_2$, IAPP 20-29) corresponding to a sequence from human IAPP which has been shown to form amyloid fibrils in vitro.[7,8] A film of the peptide IAPP 20-29 was analyzed by FTIR and shown to contain antiparallel β-sheet structure in the solid state (strong absorption at 1628 cm^{-1}).[9]

The antiparallel β-sheet, which is characteristic of amyloid, is unique among common peptide secondary structures in that extensive dipole-dipole coupling interactions occur.[9] This effect results in a splitting of the amide I band into a low-intensity band at high frequency (ca. 1695 cm^{-1}) and a diagnostic intense band at low frequency (ca. 1630 cm^{-1}).[9] The method of isotope-edited dipole coupling analysis depends on the substitution of a single amide carbonyl carbon with ^{13}C which essentially decouples that

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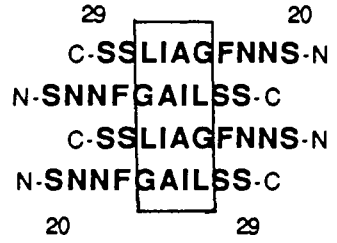

Figure 1. A crude model for a portion of the subunit antiparallel β-sheet of the IAPP 20–29 fibril which is consistent with the FTIR and ssNMR data. The enclosed section resembles the idealized antiparallel β-sheet.[4]

amide from the neighboring ^{12}C amides.[5] The effect of the substitution on the ^{12}C amide mode will depend on whether the substituted carbonyl was located in an extensively coupled (i.e., β-sheet) region of the amyloid. If the substituted amide is in the center of a β-sheet, one observes a decrease in the overall coupling of the ^{12}C amides, resulting in a decrease in the observed splitting and a shift of the intense amide I band to higher frequency. Eight analogues of IAPP 20–29 in which a single amide carbonyl was replaced with ^{13}C were synthesized[10] and analyzed in the film state by FTIR (see Table I).[11] The greatest decoupling effect was observed for analogue G24, where the ^{12}C amide peak absorbs at 1643 cm^{-1}, as compared to 1628 cm^{-1} in the natural abundance peptide (shift = +15 cm^{-1}; see Table I, column A). The magnitudes of the shifts in the ^{12}C amide absorption frequency indicate that residues G24 (+15 cm^{-1}), A25 (+9 cm^{-1}), I26 (+11 cm^{-1}), and L27 (+10 cm^{-1}) are located in the idealized β-sheet, whereas the termini of the peptide are not.

Analysis of each labeled peptide amyloid by ^{13}C cross-polarization magic angle spinning (CPMAS) solid-state NMR[12,13] revealed significant variations which are consistent with the model proposed above. Two structure-dependent variables were measured (see Table I).[13] The deviation from the "unstructured" chemical shift value[14b] (column C) and the line width (column D) are both related to structural rigidity.[14] According to each of these measurements, the region between G24 and L27 is more highly ordered than the termini of the peptide in the amyloid. The observed upfield chemical shifts (F23–L27) are typical of shifts measured for other β-sheets in the solid state.[14a]

The position of the ^{13}C amide I band for each analogue (column B) reflects the intrinsic properties of that carbonyl as well as the intermolecular dipole coupling with adjacent labeled amides in the β-sheet.[9] In order to experimentally eliminate the latter effect, each labeled peptide film was combined with five parts of the unlabeled peptide and analyzed by FTIR.[11] The observed shift in the ^{13}C band on "isotopic dilution", due to the loss of intermolecular coupling, was greatest for residues A25 (+9 cm^{-1}) and I26 (+9 cm^{-1}). The fact that intermolecular dipole coupling maximizes in the middle of the sequence is consistent with a crude model of the β-sheet in which residues A25 and I26 are proximal to one another on *both* sides of the antiparallel β-strand (see Figure 1).[15]

This model resembles the idealized cross-β fibril in the G24–L27 region.[4] The implication that this region is critical for amyloidogenesis may explain the fact that rodents, in which A25 of IAPP is substituted by proline, among other changes, do not form pancreatic amyloid.[8] Finally, the regularity of the IAPP 20–29 amyloid distinguishes it from the amyloid formed from a C-terminal fragment of the amyloid protein of AD (β34–42) which contains an unusual structural feature.[5,16]

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Supplementary Material Available: The FTIR spectra discussed herein (4 pages). Ordering information is given on any current masthead page.

(10) Purity of the ^{13}C-labeled peptides was judged to be >95% by RPHPLC (Waters Delta-Pac C4 (3.9 × 30 cm), 83% H_2O/17% CH_3CN (0.1% TFA), R_V = 23 mL). Label position was verified by fast atom bombardment mass spectrometry [$(M + H)^+$ = 1050.5]. Labeled Asn analogues were not synthesized due to expense.

(11) Thin films were formed by evaporating a formic acid (88% in water) solution of the peptide on a CaF_2 plate. Evaporation from a solution of 2:1 hexafluoro-2-propanol (HFIP)/water afforded films of nonuniform thickness; hence this solvent was not commonly used. The IR spectra of these films were identical to the formic acid films with regard to band position; however, resolution was poor due to the variability of these films.

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Cycloisomerization for Atom Economy. Polycycle Construction via Tandem Transition Metal Catalyzed Electrocyclic Processes

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Biomimetic polyolefin cyclizations have proved to be a valuable strategy for polycyclizations.[1] Mediating such processes via intermediates complexed to transition metals may offer additional avenues for control.[2] Synthetic efficiency will be enhanced if polycyclization can be accomplished by simple isomerization of some acyclic polyunsaturated species to a polycycle, with any other reagent required only in catalytic amounts.[3] We envisioned the feasibility of a stereospecific polycycloisomerization of an enediyne catalyzed by palladium (eq 1), which requires a high level of chemoselectivity in the initial hydropalladation step.

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