Amyloid–β-Sheet Formation at the Air-Water Interface

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ABSTRACT An amyloid(1-40) solution rich in coil, turn, and α-helix, but poor in β-sheet, develops monolayers with a high β-sheet content when spread at the air-water interface. These monolayers are resistant to repeated compression-dilatation cycles and interaction with trifluoroethanol. The secondary structure motifs were detected by circular dichroism (CD) in solution and with infrared reflection-absorption spectroscopy (IRRAS) at the interface. Hydrophobic influences are discussed for the structure conversion in an effort to understand the completely unknown reason for the natural change of the normal prion protein cellular (PrP^C) into the abnormal prion protein scrapie (PrP^{Sc}).

INTRODUCTION

The secondary structure conversion of the amyloid(prion)-protein in the normal form (prion protein cellular, PrP^C) into the abnormal form (prion protein scrapie, PrP^{Sc}) is the main cause of several human and animal diseases, such as the Creutzfeldt-Jakob disease (Alzheimer's), the Gerstmann-Sträussler-Scheinker syndrome, the fatal familial insomnia, the sheep scrapie, and the bovine spongiform encephalopathy (BSE) (Prusiner, 1997). PrP^C is the natural cleavage product of a larger transmembrane protein (Selkoe, 1993) and has high α-helix and low β-sheet content in contrast to PrP^{Sc}, which is rich in β-sheets (Pan et al., 1993; Safar et al., 1993). PrP^{Sc} aggregates, builds up the fibrillar filaments, and finally forms the fatal plaque deposits found post-mortem in organs and tissues (Selkoe, 1993). The reason for this helix-to-sheet transition is still completely unknown despite numerous investigations (Edenhofer et al., 1997).

Recently, Takahashi et al. (1998) proposed hydrophobic clustering as a general possibility for the protein secondary structure transformation. They modified an α-helical structured model polypeptide with a hydrophobic group at the N-terminus. The result of this substitution was a gradual change in conformation into β-sheets. These aggregated into fibrils of ~10 nm width, like the amyloid fibrils. Hydrophobic modification of the amyloid and PrP^C molecules was never found in natural systems. The β-sheet aggregation and fibril formation may be driven by interaction with surfaces. Kowalewski and Holtzman (1999) presented results of direct atomic force microscopy (AFM) observations of the aggregation of amyloid β-sheets in contact with hydrophobic graphite and hydrophilic mica. Our interest focused predominantly on the helix-to-sheet transition as the preliminary step before the aggregation and filament forming processes, and our studies were performed at the air-liquid interface. It is well-known that insoluble polypeptide and protein monolayers at the hydrophilic/hydrophobic air-liquid interface are very applicable as models for biological systems to study structure conformations and molecule orientations (Riou et al., 1998; Benjamins and van Voorst Vader, 1992; Graham and Phillips, 1979).

For investigations at the amyloidogenic protein the synthetic peptide homologous to the first 40 amino acids of the natural PrP^C-42/43 amino acids (Fig. 1) was used by a number of authors (Terzi et al., 1997; Soto and Castano, 1996; Tomski and Murphy, 1992). In our investigations we also used the amyloid(1-40). We spread a helical and random coil extensive amyloid solution at the air-water interface, and investigated the protein molecule conformation and orientation in the monolayer in situ with the Fourier-transformed IR reflection/absorption spectroscopy (FT-IRRAS). Advances in instrumentation and data processing in the last 10 years have made detailed protein analysis at the air-water interface possible, despite the unfavorable signal-to-noise ratio due to the strong absorption of the water vapor in the same wavenumber region as the proteins. A monolayer of the amyloid helices and sheets is anisotropic matter. Therefore, we have used both s- and p-polarized IR-radiation for our measurements to obtain information on the molecule orientation in the monolayer, since the IR absorption depends on the relative orientation of the incident electric field and the dipole transition moment belonging to the molecular vibrations (Cornut et al., 1996). Our goal was to study the overall structural state of the amyloid monolayer at the air-water interface, i.e., the secondary structure and the mutually molecular orientation. Different conditions, such as compression-dilatation stress alternation, interaction with trifluoroethanol, and the stability of the layer versus time were included in the investigations.

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MATERIALS AND METHODS

The amyloid(1-40) [henceforth "amyloid"] was purchased from BACHEM (Heidelberg, Germany). The purity was better than 96% (checked by HPLC). All other chemicals were obtained from SIGMA (Deisenhofen, Germany) and were of the highest purity commercially available.

1 5 10 15 20 25 30 35 40
D-A-E-F-R-H-D-S-G-Y-E-V-H-H-Q-K-L-V-F-F-A-E-D-V-G-S-N-K-G-A-I-I-G-L-M-V-G-G-V-V-I-A-T

FIGURE 1 The amino acid sequence of the amyloid peptide. The synthetic homologous 1-40 was used for the presented studies

Preparation of samples for circular dichroism and IRRAS

For circular dichroism (CD), amyloid was dissolved in 0.1% NH_3 to give solutions of 0.5 mg/ml. Aliquots were diluted with 0.1% NH_3 to the concentration of 0.25 mg/ml, resulting in an alkaline sample (pH 10). In another aliquot the pH was changed from pH 10 to pH 6.8 with 0.1 M HCl, and subsequently the sample was also diluted to the concentration of 0.25 mg/ml by adding distilled water (water resistivity >18 MΩ cm^{-1}). Furthermore, 1 mg amyloid was dissolved in distilled water and the transition of α-helix and random coil to β-sheet was executed in this solution by 3-day incubation at 37°C.

For IRRAS, 0.25 mg amyloid was dissolved in 1 ml distilled water, pH 9.5, adjusted with 0.1 N NaOH. All these stock solutions were diluted for measurement with the corresponding solvent, if necessary.

CD measurements

The CD spectra of the amyloid were recorded on a Jasco spectropolarimeter J-715 (Jasco Co., Japan) at 22°C in a 0.1-cm pathlength cell over the wavelength range 198–260 nm. The scan speed was 20 nm/min with 0.2 nm resolution. The peptide concentration was 0.1 mg/ml. The spectra were calculated from the difference in ellipticity (θ in units of deg · cm^2 · dmol^{-1}) of the peptide solution and the solvent as the reference. Percentages of the different peptide secondary structure motifs were estimated with the Selcon program using the x-ray data analysis of Levitt and Greer (see Sreeama and Woody, 1993). The method includes a tolerance of ±3%.

IRRAS measurements

IRRAS measurements were performed on the IFS 66 spectrometer from Bruker (Bruker-Saxonia, Leipzig, Germany) equipped with a liquid nitrogen-cooled MCT detector. The IR beam was conducted out of the spectrometer and focused onto the water surface of the Langmuir trough. Typically, the spectrometer, the beam path, and the trough chamber were continuously purged with dry air during spectra acquisition. The infrared beam was polarized by a polarizer (BaF_2, L.O.T. Oriel, Langenberg, Germany) in the plane of incidence (p) and perpendicular to this plane (s). Optimal conditions for detection have been obtained with an incidence angle of 70° related to the normal to the water surface. The interferograms were collected at 2 cm^{-1} resolution, apodized with a triangular function, and Fourier-transformed with one level of zero-filling2. Two thousand scans were acquired for s- and p-polarization. All reported spectra are the normalized difference of the covered and uncovered surface.

Langmuir film formation

A thermostable Teflon trough, 23 × 40 cm, equipped with a Wilhelmy plate system (filter paper 3 × 0.3 mm) and two movable motor-driven barriers (R + K, Mainz, Germany) was used and filled with distilled water as the subphase. The amyloid layers were formed by spreading 60 μl amyloid solution (concentration as above) on this surface using a Hamilton microsyringe. After 30 min the spread film was compressed to a lateral pressure of 1.5 mN/m before starting the IRRAS investigations. For one of these, 100 μl trifluoroethanol was injected into the subphase behind one of the barriers, compressed at ~30 mN/m after 1 h, and accordingly measured the IR spectra. All measurements were performed at 20°C, regulated by thermostats, in the subphase and in the vapor phase above the trough at ±1°C.

RESULTS AND INTERPRETATION

The alkaline amyloid solution examined shortly after the preparation and after standing 5 h at room temperature indicates in both cases approximately the same ratio of coil, turn, helix, and sheet. The sheet content is ~20% (Fig. 2 and Table 1). Small β-sheet content is in accordance with the natural PrP^C (Pan et al., 1993). Amyloid in vitro must be incubated for several days at temperatures ~37°C and neutral or slightly acidic pH in order to build a significant β-sheet content (Fig. 2 and Table 1). For this reason it was surprising that the alkaline amyloid solution spread at the air-water interface results in a monolayer containing a completely different ratio of the secondary structure motifs, which the IRRAS curves demonstrate in Fig. 3.

The amide I mode (essentially C═O stretching) and the amide II modes (C—N stretching, C—N—H bending) indicate the backbone secondary structure. Strong bands be-

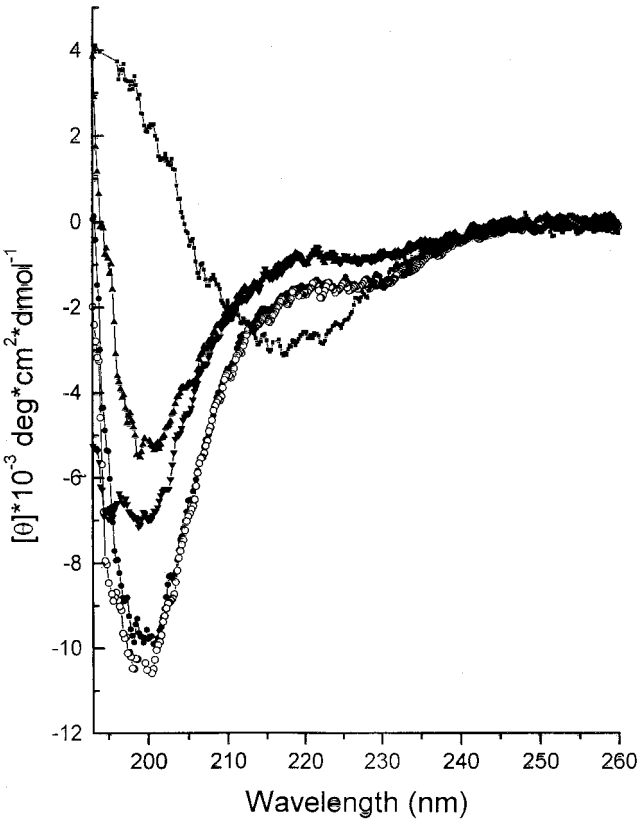

FIGURE 2 Circular dichroism (CD) spectra of amyloid(1-40) in 0.1% ammonia solution (pH 10) 10 min (▲) and 5 h (▼) after dissolution. Aliquots of the ammonia solution by change from pH 10 to pH 6.8 measured 10 min (●) and 15 h (○) later. Amyloid dissolved in pure distilled water (neutral pH) after 3-day incubation at 37°C (■). The values of the evaluated conformations are listed in Table 1.

TABLE 1 Percentage of the secondary structure motifs of amyloid(1-40) in aqueous solutions estimated by the Selcon program. See (Sreeama and Woody, 1993) for further information.

Amyloid Solution	α-Helix	β-Sheet	β-Turn	Coil
In 0.1% NH_3, pH 10				
10 min after dissolution	16	19	26	40
5 h after dissolution	14	21	25	40
Aliquots of the above alkaline solution after change from pH 10 to pH 6.8				
10 min after pH change	10	32	19	34
15 h after pH change	13	31	23	29
In pure distilled water (neutral pH) after 3-day incubation at 37°C	0	58	9	33

tween 1625 and 1640 cm^{-1} are generally taken to reflect the presence of β-sheet structure (Surewicz and Mantsch, 1993). Weaker bands around 1525 and 1680 cm^{-1} can also be assigned to β-sheet. The formation of parallel and antiparallel β-sheet strands produces the band-splitting of the amide I mode in the region around 1630 cm^{-1}. In conformity with investigations of amyloid in aqueous samples (Fabian et al., 1993; Caŭghey et al., 1991) the broad band around 1535 cm^{-1} and the weaker bands near 1563 and 1650 cm^{-1} can be assigned to the α-helix, admittedly not separable from the irregular random coil state in any case.

The top curve in Fig. 3, measured with the p-polarized IR beam, manifests α-helix, random coil, and parallel and antiparallel β-sheets, all of them in comparable amounts. However, a difference in the reflectance/absorption between the two polarizations is striking: the dominant signal appears in the s-polarized beam, indicating antiparallel β-sheets. This means the amyloid monolayer at the interface is composed mainly of antiparallel β-sheets arranged in the plane of the water surface. Only very small amounts of

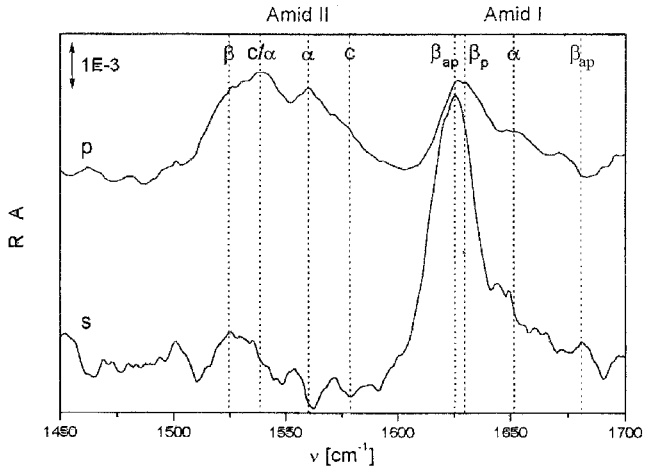

FIGURE 3 IRRAS curves of the amyloid(1-40) monolayer at the air-water interface measured in the p- (*top*) and s- (*bottom*) polarized IR-beam. The monolayer was produced by spread of an alkaline aqueous solution of amyloid (pH 9.6) at the interface. The indicated symbols mean helix (α), parallel and antiparallel β-sheet (β_p, β_{ap}), and random coil (C).

α-helix, random coil, and parallel β-sheets exist in this plane. These structures, together with a small content of the antiparallel β-sheets, are mainly orientated perpendicular to the surface. The β-sheet-enriched monolayer is invariant toward compression-dilatation stress alternation. This results from the following investigations: the spread monolayer was compressed to a lateral pressure of 13 mN/m, kept at this level for 30 min, and afterward released to about zero mN/m. This cycle was executed altogether three times, in each case after an intermission of 30 min.

The IRRAS curves in Fig. 4 *a* show the different compression and dilatation steps and indicate a high stability of the β-sheet-enriched amyloid layer at the air-water interface. Indeed, the difference spectra of successive steps (Fig. 4, *b* and *c*) seem to accentuate increased antiparallel β-sheet, formed essentially at the expense of coils and helices, which were oriented as well in parallel as in perpendicular alignment to the surface plane. However, these effects can also be reorientations of the molecules, because no additional bands corresponding to another secondary structure are observed. This is also supported by the results of a monolayer aged 24 h (Fig. 5 *a*). In the difference spectra (Fig. 5, *b* and *c*) one observes the increase of β-sheets in s-polarization and their decrease in p-polarization (comparable magnitude) more clearly. From that we conclude stress alteration and aging of the monolayer can cause molecular orientation changes, but not a conformation transition of the secondary structure, because the amyloid monolayer rich in β-sheets has a great conformation stability. Furthermore, trifluoroethanol, which supports the development of α-helices in general (Terzi et al., 1997; Takahashi et al., 1998), was not able to change the conformation of the monolayer. The trifluoroethanol injected into the subphase penetrated into the spread and compressed the amyloid monolayer. That was visible in the π/A-isotherm in an increase of the area/amyloid-molecule values upon trifluoroethanol penetration and under the Brewster-angle microscope (BAM) from an increasing spongy packing of the monolayer (isotherm and BAM picture not shown). Despite this trifluoroethanol penetration an essential change of the secondary structure of the spread amyloid monolayer was not detectable by IRRAS (Fig. 6).

DISCUSSION

The central message of this work is that a stable β-sheet-enriched state of the amyloid is formed at the air-water interface, in contrast to the initial bulk solution containing high α-helix/random coil and low β-sheet parts. What is the reason for this secondary structure conversion? The change in the pH going from bulk (alkaline pH) to the interface (neutral or slightly acidic pH) can have effects on the conformation at the interface, because our CD measurements in the bulk indicate that in changing pH from 10 to 6.5, the β-sheet content increased from ~20 to 32% (Table 1). Davidson and Fasman (1967) have demonstrated with

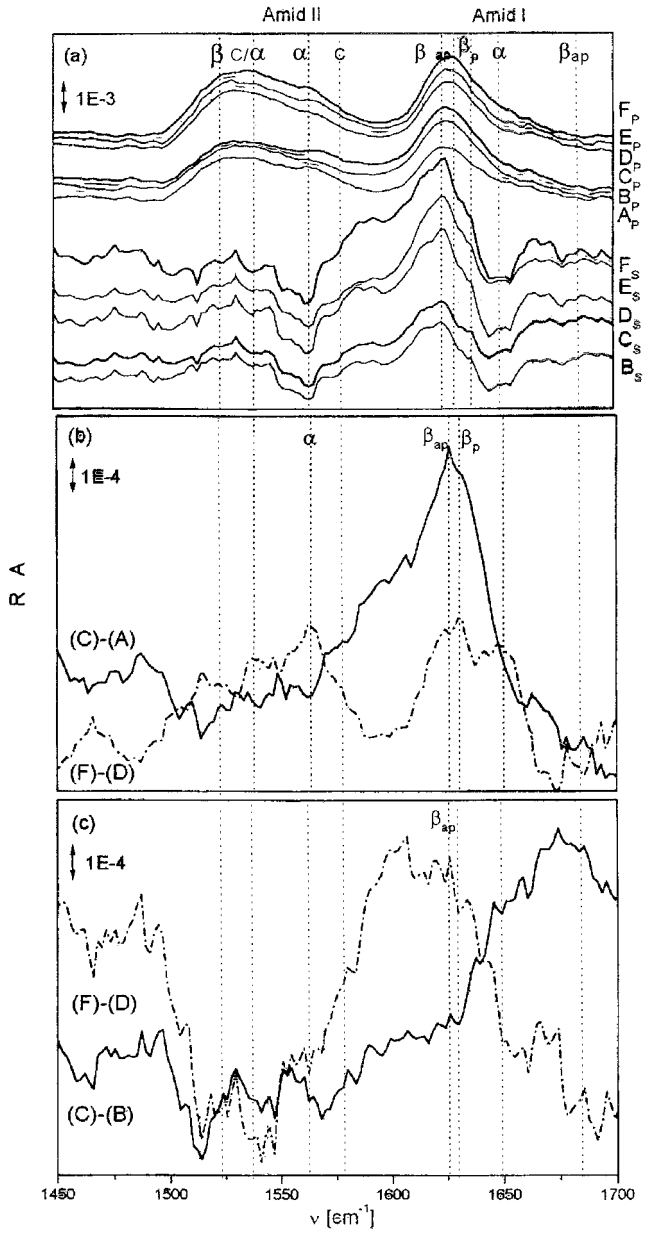

FIGURE 4 IRRAS curves of the amyloid(1-40) monolayer at the air-water interface. The monolayer was compressed to a lateral pressure of 13 mN/m for 30 min and released to 1.5 mN/m three times in succession. In (*a*), A–C and D–F are the monolayers at the lateral pressure of 1.5 mN/m and 13 mN/m, respectively, measured in the p- and s-polarized beam (indicated by index p, s). A/D, B/E, and C/F are the corresponding curves in the first, second, and third compression/dilatation cycle. The curves in (*b*) and (*c*) indicate corresponding difference spectra measured in the p- (*b*) and s- (*c*) polarized IR beam, respectively.

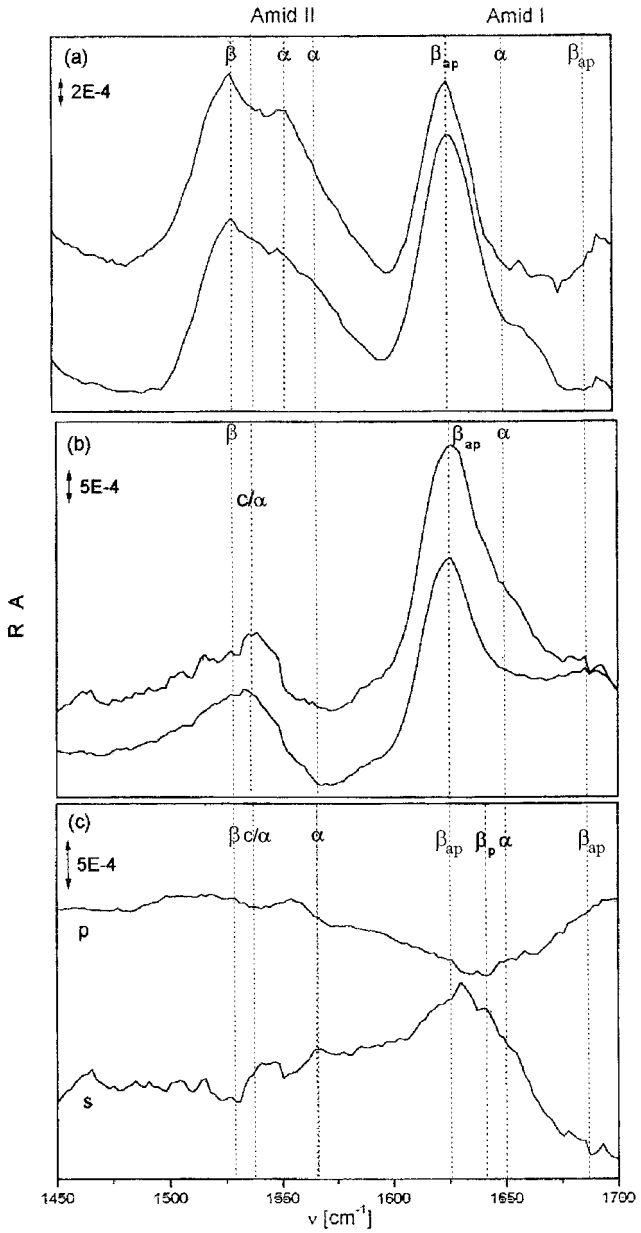

FIGURE 5 Aging of the amyloid monolayer at a lateral pressure of 10 mN/m for 24 h. IRRAS curves were measured in the p- (*a*) and s- (*b*) polarized IR beam. The top curves in (*a*) and (*b*) indicate the start condition, and the bottom curves were measured after 24 h. Diagram (*c*) exhibits the difference between these two times.

polylysine that formation and stability of the β-sheet conformation can be owed partly to interactions between hydrophobic segments of the residue side chains. The greatest effect takes place if the side chains are uncharged, which is well-served at the isoelectric point. The isoelectric point of amyloid is at pH 5 (Esler et al., 1996) and the amyloid drifts in this direction by spreading the alkaline solution at the pure water interface. However, this pH effect cannot be the exclusive reason for the conformational change, because the β-sheet has increased from ~20 to 32% in solution by this pH change only, but at the interface the β-sheet content has grown to ~75%. This value can be estimated by comparing the main absorption planes of the secondary structure motifs in Fig. 3. Therefore, in addition to the pH change another effect must play a role. Rearrangement in the bulk, e.g., by incubation at 37°C, typically requires many days, whereas at the interface the structural change was observed after minutes at 20°C. Therefore, another alternative might be the intrinsic hydrophobicity of the air-water interface, which is

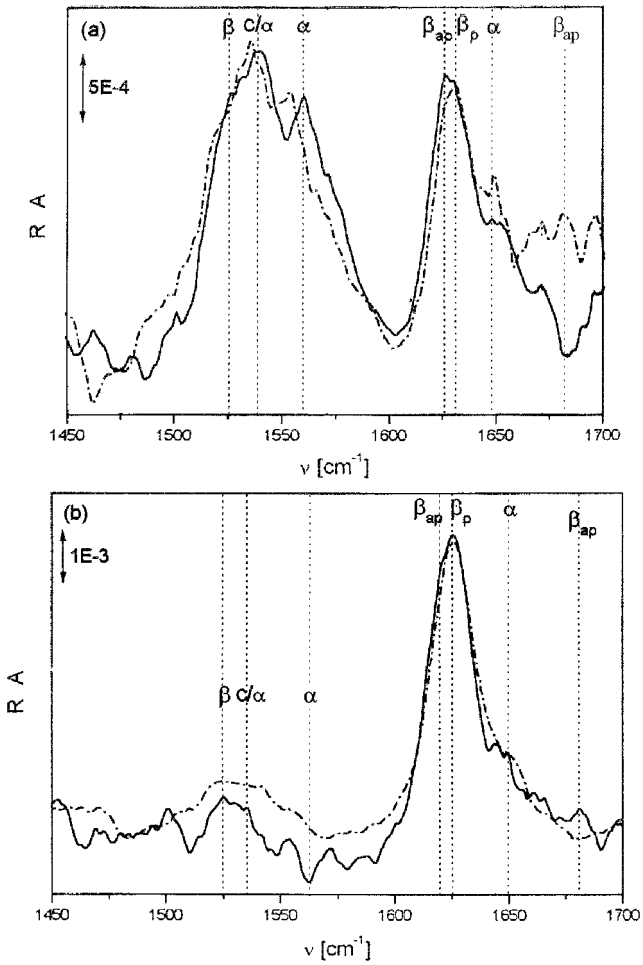

FIGURE 6 IRRAS curves of the amyloid(1-40) monolayer at the air-water interface, measured in the p- (*a*) and s- (*b*) polarized IR beam before (*solid line*) and after (*dashed line*) injection of trifluoroethanol into the subphase.

a hydrophobic-hydrophilic system with air as the hydrophobic part.

Generally, hydrophobic effects of protein-extraneous components changing protein secondary structures are well-known and have been found on various occasions, such as with hydrophobically coated quartz plates (Wu et al., 1993) and by peptides, substituted with the hydrophobic adamantane (Takahashi et al., 1998). Wu et al. observed a loss of helical and coil structure with time and an increase in β-sheets for different globular proteins adsorbed on hydrophobic quartz. Takahashi et al. have shown that peptides folded in an α-helix structure can change gradually to the β-sheet structure, in aqueous solution, if modified with a hydrophobic adamantane group at the N-terminus. The pathway of this transformation is still poorly understood and cannot satisfactorily explain the predominance of the β-sheets in the present case of amyloid at the air-water interface. The partially hydrophobic nature of the amyloid molecule seems to be, in this case, the feature for the structure transformation at the air-water interface.

The amyloid has two hydrophobic clusters in their amino acid sequence: position 17–21 and 29–40, the last only interrupted by methionine in position 35 (see Fig. 7, *underlines*). In an aqueous bulk phase these hydrophobic side chains are packed inside the structure, which is possible in the helix or the random coil. At the air-water interface the hydrophobic side chains can be exposed to the hydrophobic air, which is possible only as sheet under the supposition that the sheet is arranged parallel to the interface (Fig. 7 *a*). As an alternative one might assume the β-sheet can stretch into the air. This, however, is only possible if there is a lateral interaction, i.e., in a lipid monolayer. The present IRRAS measurements suggest the parallel arrangement in principle. Divergences from the parallel β-sheet orientation toward the perpendicular direction are forced by the α-helical structured sections within the amyloid molecule (Fig. 7 *c*). For the first step of the amyloid-sheet arrangement at the air-water interface one might assume that hydrogen bonds of interface-water molecules stabilizing the single sheet strand are formed at the interface. This is known for the pure water surface (Lee et al., 1984) and may be the case here, too. Thereupon, in a second step these single strands interconnect to the parallel and mainly antiparallel β-sheets by intermolecular hydrogen bonds.

Natural biological processes do not happen at the air-water interface, but in the blood and lymph liquid at membrane interfaces, surfaces of protein-, enzyme-, DNA-colloids, and so on. Knowledge about the secondary structure behavior of the amyloid at these conditions is still poor. Terzi et al. (1997) have distinguished two well-defined equilibria for human amyloid upon titration with phospholipid vesicles: a coil/β-sheet equilibrium at low lipid-to-protein ratios that is followed by a transition to helices at ratios >55. Because a penetration of the peptide into the vesicle interior was excluded, the helix formation seems to be probably triggered by electrostatic interactions at the vesicle interface. Biomembranes and the other natural biosystems are hydrophilic systems, but the interior biological interfaces also have parts of a more or less hydrophobic nature. Furthermore, hydrophobic deposits of various types can happen at these membranes during the vital processes.

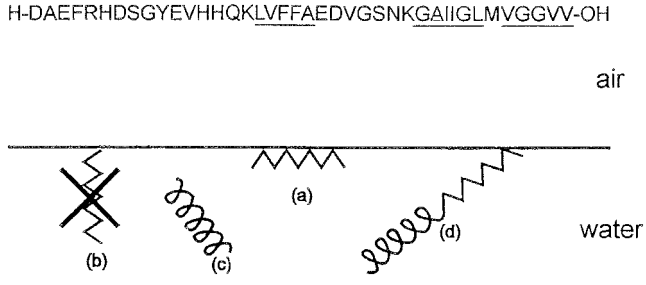

FIGURE 7 Scheme of the different possibilities for the amyloid secondary structure arrangement at the air-water interface: β-sheet orientated parallel to the interface (*a*); perpendicular to the interface (not possible) (*b*); α-helix in the interface-water section (*c*); and an $\alpha\beta$-unit (*d*). The hydrophobic clusters in the amyloid sequence are underlined.

These could be good places for the structural conversion of amyloid to β-sheets in the demonstrated manner caused by hydrophobic effects at a membrane interface and could be a model for the natural PrP^{C}-to-PrP^{Sc} transition.

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