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Solution NMR of proteins within polyacrylamide gels: Diffusional properties and residual alignment by mechanical stress or embedding of oriented purple membranes

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Abstract

The diffusive properties of biomacromolecules within the aqueous phase of polyacrylamide gels are described. High quality NMR spectra can be obtained under such conditions. As compared to water, a fivefold reduction in the translational diffusion constant, but only a 1.6-fold decrease (1.4-fold increase) in amide-^{15}N T_2 (T_1) are observed for human ubiquitin within a 10% acrylamide gel. Weak alignment of the solute macromolecules can be achieved within such gels by vertical or radial compression or by the embedding of magnetically oriented purple membrane fragments. The methods are applied to derive residual dipolar couplings for human HIV-1 Nef and ubiquitin.

Introduction

High resolution NMR spectra of peptides bound to resin beads can be obtained by the use of magic angle spinning (MAS) (Warrass et al., 2000). In these systems, MAS is needed to average out field inhomogeneities introduced by the strongly discontinuous bead/solvent interface. This prompted us to characterize the spectral properties of biomacromolecules dissolved in aqueous solution within polyacrylamide gels where such discontinuities are absent. In this paper, we describe the diffusive properties of biomacromolecules in such a system and show that high resolution NMR spectra can be obtained without the use of MAS. Furthermore, weak alignment (Tolman et al., 1995; Tjandra and Bax, 1997) of the solute molecules can be achieved within the aqueous phase of such a gel by a variety of methods such as mechanical stress of the gel or by the embedding of magnetically oriented purple membranes (Lewis et al., 1985). Independent work describing the mechanical alignment within gels has recently been published by Tycko and co-workers (Tycko et al., 2000). Both mechanical and purple membrane alignment are well suited for the practical measurement of residual tensorial couplings. Clearly, the polyacrylamide system is chemically and mechanically very stable. Its orientation is not dependent on the magnetic field such that the direction of the alignment can be varied with respect to the field axis. The possibility to obtain high resolution NMR spectra of freely diffusing biomacromolecules within such a gel also opens the stage to study interactions of biomacromolecules with other functional groups or molecules that are attached to the rigid matrix of the gel.

Methods

The following procedure proved to be robust to fill NMR tubes homogeneously by the gels and to in-

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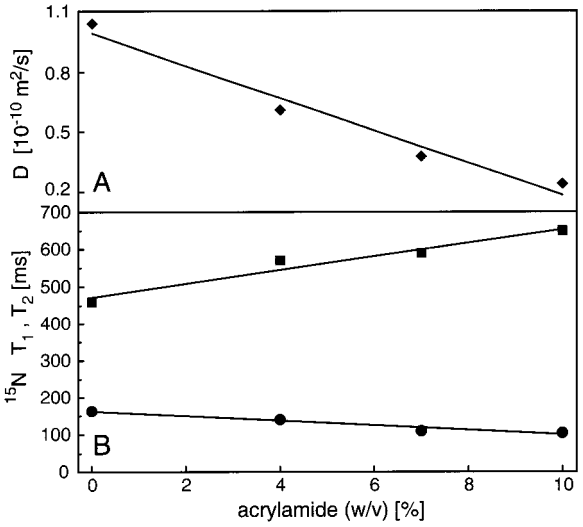

Figure 1. Translational and rotational diffusion properties of ^{15}N-labelled ubiquitin within polyacrylamide gel as a function of gel density. (A) Translational diffusion coefficients D. The diffusion coefficient for each gel density was determined from a series of experiments with four gradient strengths (G = 11, 22, 33, 44 G/cm) using a modified PFGLED sequence (Dingley et al., 1995). (B) Average amide-^{15}N $^{1}T_1$ (rectangles) and T_2 (circles) relaxation times as determined from one-dimensional ($^{1}H^{N}$-detected) versions of the respective heteronuclear experiments (Kay et al., 1992).

troduce proteins into the aqueous phase within the gels at the same time: a cylindrical, glass or plastic tube of suitable diameter (3.5 to 8 mm, see below) is closed on one end by parafilm. An acrylamide solution is prepared at the desired concentration by aqueous dilution from a stock of 29.2% w/v acrylamide and 0.78% w/v N,N′-methylenebisacrylamide. An appropriate volume of the acrylamide solution is filled into the tube, just after the polymerization has been started by the addition of 0.1% w/v ammonium persulfate and 0.5% w/v tetramethylethylenediamine (TEMED). After polymerization is complete, the gel can be pushed out from the tube by the application of gentle mechanical pressure. After washing the gel is dried on a smooth plastic support at 37 °C in a drying oven for several hours such that its diameter shrinks to about 1–2 mm. The dried gel is then placed into an NMR sample tube and a buffer solution containing a suitable concentration of macromolecules is added. After 3–4 h of swelling, the gel has usually filled out the sample tube and high resolution NMR measurements can be performed.

Results and discussion

Determination of solute diffusion parameters

In order to characterize the diffusion of proteins within the gel phase, translational diffusion coefficients D as well as average ^{15}N-T_1 and ^{15}N-T_2 relaxation times were determined from one-dimensional spectra of samples containing 0.25 mM ^{15}N-labelled ubiquitin, 10 mM phosphate, pH 7.0, 95% H_2O, 5% D_2O at 20 °C in the absence of gel and within 4, 7, and 10% (w/v) polyacrylamide gels (Figure 1). These gels had been cast with an original diameter which was identical to the inner diameter of the sample tube. Clearly, close to linear dependencies on the gel density are observed for all three parameters, D, ^{15}N-T_1, and ^{15}N-T_2, which follow the expected trends that translational and rotational diffusion are slowed down within the acrylamide matrix. The higher viscosity in the aqueous pores of the gel results in a decrease of the translational diffusion coefficient by a factor of about 5 from 1.06 to 0.23 10^{-10} m^2/s for the change from 0 to 10% acrylamide (Figure 1A). The rotational correlation time of ubiquitin, however, is not affected to the same extent: compared to water, the presence of 10% gel results only in an increase of T_1 by a factor of 1.41 (from 0.46 to 0.65 s) and a decrease of T_2 by a factor of 1.55 (from 163 to 105 ms; Figure 1B). The close agreement of the relative increases and decreases of T_1 and T_2 hints at a true slowing of the rotational diffusion within the gel and precludes significant contributions from chemical exchange broadening. Neglecting more subtle effects of anisotropic diffusion (Tjandra et al., 1995), which could only be analyzed on a per residue basis from two-dimensional relaxation experiments, we estimate that the isotropic rotational correlation time for ubiquitin is increased from approximately 5 ns at 20 °C in aqueous solution to 7–8 ns within the 10% acrylamide gel. The different scaling behavior of translational and rotational diffusion constants with respect to the increase in gel density is not surprising, considering the heterogeneous microstructure of a gel and the different length scales involved in the two processes. Whereas rotational diffusion occurs on a scale of the solute size, i.e. about 3 nm, translational diffusion as detected by the magnetic pulsed field gradient experiments occurs on the μm scale.

For small molecules such as ubiquitin, the observed 70% decrease in T_2 within the 10% gel is not really prohibitive for recording high quality NMR spectra. For larger proteins, however, lower gel den-

Table 1. Characterization of ubiquitin and HIV-1 Nef for different residual alignment preparations

Sample[a]	ubi. 1	ubi. 2	ubi. 3	ubi. 4	ubi. 5	ubi. 6	ubi. 7	ubi. 8	Nef 1	Nef 2
Medium	4% paa[b]	7% paa	10% paa	4% paa	6% paa	DMPC/DHPC[c]	PM + 4% aa[d]	PM + 4% paa[e]	4% paa	7% paa
Length ratio[f]	0.77	0.80	0.78	0.86	0.86	–	–	1.0	0.75	0.75
Diameter ratio[g]	1.17	1.17	1.17	0.51	0.73	–	–	1.0	1.17	1.17
Anisotropic compression[h]	0.66	0.68	0.67	1.67	1.18	–	–	1.0	0.64	0.67
D [10^{-11} m^2s^{-1}]	6.1	4.0	2.8	2.3	2.8	nd[i]	nd	4.5	nd	nd
^{15}N-T_1 [s]	0.54	0.61	0.64	0.80	0.76	nd	nd	0.62	0.95	1.05
^{15}N-T_2 [ms]	134	110	96	90	86	nd	nd	98	55	45
A^j_{2r}	−0.00	−0.50	−1.33	0.69	0.56	0.32	0.19	0.72	0.36	3.79
A_{2i}	0.37	2.57	5.41	−1.54	−2.00	−2.81	6.94	4.62	0.32	1.21
A_{1r}	1.03	5.06	10.36	−2.99	−3.78	−6.73	4.17	4.09	−0.81	−6.78
A_{1i}	0.05	0.57	1.25	−0.22	−0.28	−4.17	−4.59	−4.99	−0.99	−13.88
A_0	0.89	4.43	8.90	−3.59	−3.55	−9.05	0.97	0.20	−0.81	−18.20
A^k_{zz}	1.07	5.35	10.88	−3.45	−4.02	−9.35	−8.25	−7.11	−1.16	−17.89
η^l	0.60	0.75	0.79	0.82	0.83	0.22	0.23	0.03	0.97	0.37
rmsd[m] [Hz]	0.27	0.59	1.32	0.64	0.76	0.75	1.38	1.20	1.01	3.61
N[n]	56	56	57	52	52	41	52	50	66	56

[a] Sample conditions: ubi. 1–5: 0.25 mM ^{15}N-labelled ubiquitin, 10 mM phosphate, pH 7.0, 20 °C; ubi. 6: 0.25 mM ^{15}N-labelled ubiquitin, 30 mM phosphate, pH 7.6, 40 °C (data are taken from Sass et al. (1999)); ubi. 7,8: 0.25 mM ^{15}N-labelled ubiquitin, 30 mM phosphate, pH 7.0, 95% H_2O/5% D_2O, 20 °C. Nef 1,2: 0.4 mM ^{15}N-labelled HIV-1 Nef$^{\Delta 2-39}$(Grzesiek et al., 1997), 20 mM Tris, pH 8.0, 95% H_2O/5% D_2O, 35 °C. For samples ubi. 1–3 and Nef 1–2 orientation was induced by vertical squeezing (procedure 1), for ubi. 4,5 by radial squeezing (procedure 2), for ubi. 6 by DMPC/DHPC, for ubi. 7,8 by oriented purple membranes.

[b] Polymerized acrylamide.

[c] Suspension (3% (w/v)) of DMPC/DHPC 3.1:1 (w/w).

[d] 4 mg/ml purple membrane + 4% acrylamide solution before polymerization.

[e] 4 mg/ml purple membrane + 4% acrylamide solution after polymerization in the magnet.

[f] Final length of the gel (in the sample tube)/initial length (after polymerization). Typical initial lengths were 35 mm.

[g] Final diameter of the gel (in the sample tube, i.e. 4.1 mm)/initial diameter (after polymerization).

[h] Length ratio/diameter ratio.

[i] Not determined.

[j] $A_{mr,i}$ real and imaginary parts of the irreducible alignment tensor (Sass et al., 1999). Values are given in Hz. The dipolar coupling for an individual amide NH vector is calculated from these values according to the formula: $D_{NH} = \Sigma_{m=-2,2}\ A^*_m Y_{2m}(\theta,\phi)$, where θ and ϕ are the polar angles of the NH vector, Y_{2m} are second order spherical harmonics and $A_{-m} = (-1)^m A^*_m$.

[k] z-component of Cartesian alignment tensor in the principal axis system. $D_{NH} = A_{zz}((3\cos^2\theta - 1)/2 + \eta/2\ \sin^2\theta\ \cos 2\phi)$.

[l] Rhombicity.

[m] Root mean square deviation of theoretical and calculated dipolar couplings.

[n] Number of residues used in the fit. Only residues with low internal mobility were considered.

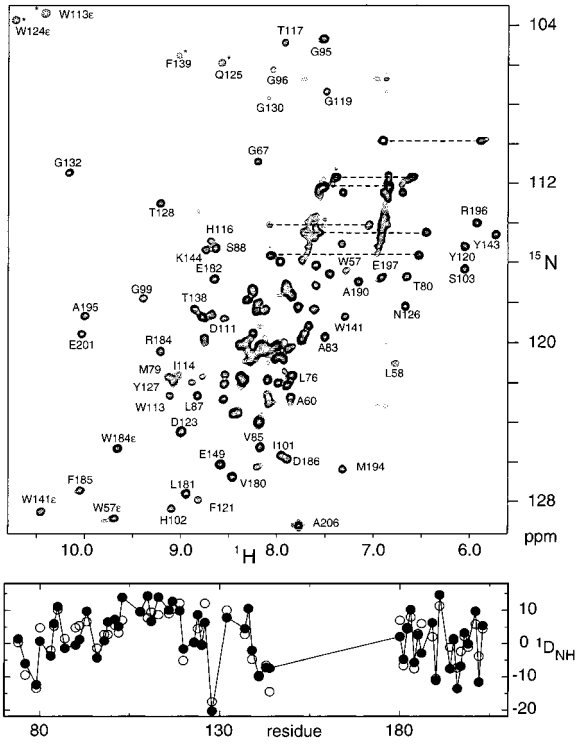

Figure 2. Top: ^{1}H-^{15}N HSQC of HIV-1 Nef$^{\Delta 2-39}$ (Grzesiek et al., 1997) dissolved in the aqueous phase of a 4% polyacrylamide (Nef sample 1 in Table 1). Total acquisition time 2 h. Peaks are labeled with assignment information. Resonances marked by asterisks are folded in the ^{15}N dimension. This and all other spectra were recorded on a Bruker DMX-600 NMR spectrometer equipped with a triple-axis pulsed field gradient ^{1}H/^{15}N/^{13}C probehead optimized for ^{1}H detection. Bottom: measured (filled circles) and calculated (open circles) dipolar couplings for Nef sample 2 (Table 1, 7% polyacrylamide, vertical squeezing) as a function of residue number. HN bond vector orientations were derived from the 1AVV X-ray structure (Arold et al., 1997).

Table 2. Correlation coefficients between the irreducible components of alignment tensors for various samples of oriented ubiquitin

Sample[a]	2	3	4	5	6	7	8
1	0.99[b]	0.99	−0.97	−0.99	−0.91	0.60	0.57
2		1.00	−0.99	−1.00	−0.93	0.63	0.57
3			−0.99	−1.00	−0.92	0.63	0.57
4				0.99	0.93	−0.59	−0.53
5					0.92	−0.64	−0.58
6						−0.33	−0.24
7							0.97

[a] Sample numbering refers to ubiquitin samples of Table 1.

[b] The correlation coefficient r is calculated as the normalized scalar product of the irreducible components of two orientation tensors $<A^1|A^2>/(<A^1|A^1>^{1/2}<A^2|A^2>^{1/2})$ with $<A^1|A^2> := \Sigma_{m=-2,2}\, A^1_m A^{2*}_m$.

sities are preferable. Figure 2 shows as an example a ^{1}H-^{15}N HSQC of a 0.4 mM solution of HIV-1 Nef$^{\Delta 2-39}$ (MWT 19.5 kDa) obtained in a 4% (w/v) polyacrylamide/water gel at 35 °C. As expected, the transverse relaxation times are also reduced to some extent (20–30%) for this protein by the presence of the polyacrylamide matrix. However, the spectral quality is sufficiently high to resolve most of the amide resonances into non-overlapping cross peaks (Figure 2). The gel drying and soaking procedure described above is limited to gels with higher densities than about 4%. Below 4%, the gels are mechanically very unstable. In order to introduce macromolecules into such low density gels, polymerization in the presence of the macromolecular solution might be an alternative (see below).

As judged from the line widths in ^{1}H-^{15}N HSQCs, effects of inhomogeneous line broadening on the ^{1}H resonances of the solute macromolecules in such unstrained gels appeared to be very modest (< 2 Hz). This indicates that the unstrained gels had filled the sample tube in a rather homogeneous way. However, an increase in the inhomogeneous line broadening on the order of 5 Hz was observed when mechanical stress was applied to the gels by either one of the two procedures described below.

Solute alignment by mechanical stress of the gel

The possibility to record NMR spectra of macromolecules in the gel phase cannot only be used to study macromolecules and their interactions within such a restrained medium or to explore macromolecular interactions with gel-embedded or attached substances. It can also be used to study the behavior of macromolecules when macroscopic forces, such as mechanical pressure or electric fields are applied to the gel. In this way, for example, weak, second-rank alignment can be achieved by mechanical stress. Two procedures proved to be robust for obtaining such an orientation: (1) non-isotropic (vertical) pressure can be applied by pushing the plunger of a Shigemi sample tube onto the gel. After the dried gel and the aqueous macromolecular solution have been placed into the sample tube, the pressure from the plunger is applied during the swelling process when the gel has reached a diameter which is slightly smaller than the inner diameter of the tube. In the absence of such a small cleft between the gel and the sample tube wall, vertical compression by the plunger becomes difficult. In contrast, if the gel diameter is considerably smaller than the inner diameter of the tube, the application of vertical pressure

leads to a coiling of the gel and a very inhomogeneous filling of the tube. Best results were obtained when the original gels were cast at a diameter which was slightly smaller (∼0.5 mm) than the inner diameter of the sample tube. However, also larger original diameters can be used which result in an increase in gel density by the additional radial restriction from the sample tube walls (see below). (2) Radial pressure can be applied by the following procedure: the gel is originally cast in a cylindrical tube of a larger diameter than the sample tube. During the reswelling of the dried gel no vertical pressure is applied. This results in a stronger radial than vertical compression of the gel. For this procedure, it proved beneficial to coat the inner surface of the tube by dimethyldichlorosilane such that the gel can slide more easily along the tube walls during swelling. The different strains resulting from the two procedures are expected to produce different anisotropies within the gel. Procedure 1 should lead to oblate cavities with their normal in the direction of the tube, whereas procedure 2 should lead to prolate cavities.

Figures 3A and B show observed and calculated residual dipolar couplings $^1D_{HN}$ for human ubiquitin which were obtained by gel strain procedures 1 and 2, respectively. Calculated values of $^1D_{HN}$ were derived by a linear fit of the irreducible components of the orientation tensor (Sass et al., 1999) to the 1.8-Å X-ray structure of ubiquitin (Vijay-Kumar et al., 1987) and the experimental data. For these and other preparations, the orientation tensor, fit quality, diffusion coefficients, relaxation times, as well as geometric or chemical parameters of the orienting gels are listed in Table 1. Clearly visible in Figure 3A,B are residual dipolar couplings in the range of ±6 Hz which were obtained at acrylamide gel densities of 6–7% (samples ubi. 2 and 5 in Table 1). Apparently, the sign of the orientation tensor (Table 1) is simply inverted between the two procedures. This is exemplified by residues D52 and T22 which have the largest and smallest dipolar splittings, respectively, for the vertical compression procedure 1 (Figure 3A) whereas they show the opposite behavior for the radial compression procedure 2 (Figure 3B). The collinearity of both orientation tensors is more quantitatively described by the correlation coefficient r (Table 2) of their irreducible components (Sass et al., 1999). For samples ubi 2 and 5, this correlation coefficient has a value of −1.00, indicating complete linear dependence. Listed in Tables 1 and 2 are also data for the orientation tensor of ubiquitin in the presence of DMPC/DHPC bicelles (sample

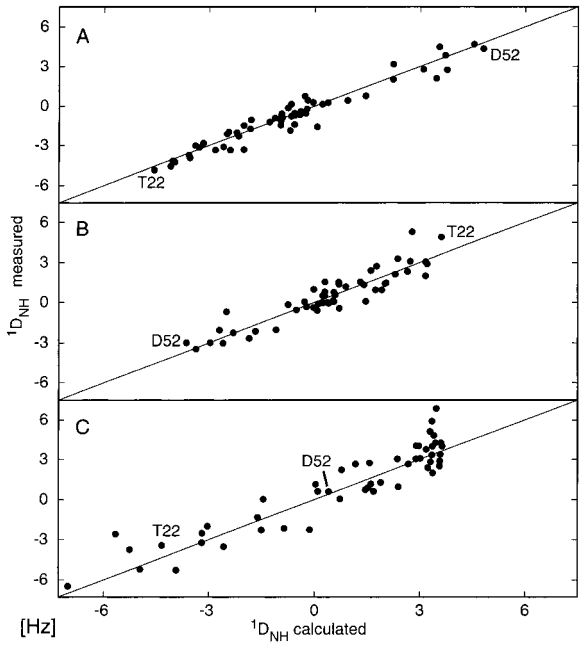

Figure 3. Measured and calculated dipolar couplings $^1D_{HN}$ for ubiquitin under varying conditions of gel-induced alignment. Measured values of $^1D_{HN}$ were derived from 0.5 h doublet-separated sensitivity-enhanced HSQC experiments with ^{15}N-decoupling during data acquisition (Cordier et al., 1999) and a comparison to $^1J_{HN}$ data in the isotropic state. Coupling constants for residues T22 and D52 are marked in order to illustrate differences in the alignment tensors. (A) Ubiquitin sample 2 (see Table 1) in 7% polyacrylamide using alignment by vertical squeezing of the gel (procedure 1). (B) Ubiquitin sample 5 in 6% polyacrylamide using alignment by radial squeezing (procedure 2). (C) Ubiquitin sample 8 in 4% polyacrylamide with embedded magnetically oriented purple membranes (4 mg/ml).

ubi. 6). This orientation tensor is almost parallel (r = 0.92) to the tensor of the radially squeezed gels and almost antiparallel (r = −0.93) to the vertically squeezed gels. Apparently the orienting forces in the gels are very similar to the steric hindrance observed in the DMPC/DHPC bicelle system (Tjandra and Bax, 1997). It is worthwhile to mention that the small deviation from complete collinearity of the tensors in both media is significant and results in a change of the rhombicity from 0.23 in the bicelles to about 0.8 in the gel systems (Table 1). For ubiquitin, it is noted that for a similar size of the orientation tensor A_{zz}, the rmsd between observed and calculated couplings is about 1.8 times smaller for the DMPC/DHPC system (ubi. 6) than for the gel system (ubi. 3). This effect can be attributed to statistical errors resulting from a reduction in sensitivity of the gel experiment because the transverse relaxation times in the 10% gel phase

at 25 °C are more than twofold shorter than in the DMPC/DHPC system at 40 °C.

Table 1 also lists orientation tensors obtained by procedure 1 for gel concentrations of 4% (ubi. 1) and 10% (ubi. 3) and by procedure 2 for a 4% gel (ubi. 4). In all cases the orientation tensors are very close to collinear (Table 2). For samples ubi. 1–3, which were prepared under identical conditions of vertical compression (procedure 1), the size of the orientation tensor A_{zz} clearly increases monotonically with the gel density (Table 1). For these samples, the total volume of the strained gel is nearly identical to the volume of the original gel because the vertical compression is compensated by an equivalent lateral expansion. In this case, the translational diffusion coefficients as well as the ^{15}N relaxation times (Table 1) are very close to the values obtained within the unstrained 4, 7 and 10% gels (Figure 1). In contrast, friction at the sample tube wall and the limited plasticity of the gel hinder the vertical expansion during swelling in the radial compression procedure 2 (Table 1) such that a significant increase in gel density results. Therefore, the translational diffusion coefficients and ^{15}N relaxation times for samples ubi. 4 and 5 (procedure 2, original densities 4 and 6%) are closer to values expected for unstrained gels of 10–15% density. For this reason, procedure 1 seems more suitable for samples where transverse relaxation times are a limiting factor.

Orientation according to procedure 1 was also applied to the HIV-1 Nef protein at 4 and 7% gel densities (Table 1). Best results were obtained at 7% gel density (Figure 2, bottom), which yielded a very appreciable size of the orientation tensor A_{zz} of -17.9 Hz albeit at a reduction in amide ^{15}N T_2 from about 70 ms in aqueous solution to 45 ms (Table 1). Nevertheless, the quality of the spectra was sufficient to unambiguously derive 64 $^1D_{NH}$ couplings within the backbone of the HIV-1 Nef core. This result is particularly appreciable because it was not possible to obtain high quality residual couplings for this hydrophobic protein by other methods of orientation such as the lipid bicelle or the purple membrane system (G.M., unpublished data). It is revealing to compare the dipolar coupling data to both the NMR solution structure [2NEF (Grzesiek et al., 1997)] and the 3.0 Å resolution X-ray structure [1AVV (Arold et al., 1997)]. The rmsd of heavy atom backbone positions between the non-mobile elements of both structures is smaller than 1.5 Å. However, the NMR quality factor (Cornilescu et al., 1998) indicates a significantly better agreement with the X-ray structure (0.45 versus 0.68). A preliminary analysis shows that these deviations are caused to a large extent by a slight rearrangement of the second alpha helix. This finding is not surprising since the NMR structure was derived from a limited amount of long-range NOE data, a rather extensive number of scalar coupling dihedral restraints, but without the use of any dipolar couplings. Therefore long-range order between secondary structure elements was less well defined than the local geometry of individual amino acids. Incorporation of the dipolar restraints is expected to improve the quality of the NMR structure significantly.

Solute alignment by embedding of preoriented substances

As an alternative method of residual orientation of solute macromolecules within the gel phase we have explored the possibility to obtain such an anisotropy by the interaction with magnetically aligned purple membranes which had been embedded into the polymerized gel. Due to the high anisotropy of their magnetic susceptibility, purple membrane fragments are aligned by the magnetic fields of current NMR spectrometers (>10 T) to almost 100% with their normal pointing in the direction of the magnetic field (Sass et al., 1999). This orientation can be made permanent when the purple membrane fragments are suspended in an aqueous acrylamide solution and the polymerization is carried out within the magnet (Dresselhaus, 1988).

In principle, it is possible to introduce solute macromolecules into such gels by perfusion with a suitable macromolecular buffer solution. However, due to the restricted translational diffusion, this process is very slow. Complete perfusion by ubiquitin of a 4% gel containing 4 mg/ml of magnetically aligned purple membrane was reached only after about 2 weeks when the gel was polymerized in an NMR sample tube and ubiquitin was introduced by topping the gel with a 2 mM ubiquitin solution. On this system, residual dipolar coupling on the order of 8 Hz could be detected (data not shown). Faster perfusion can be obtained by drying and resuspension of the gels as described above. However, this seemed to destroy the alignment of the purple membranes. An even faster and simpler method to introduce the macromolecules into the gels consists in carrying out the gel polymerization reaction within the magnet already in the presence of the solute macromolecule. Surprisingly, the intensity of the NMR resonances of ubiquitin was

only reduced by about 20–30% during this procedure. This indicates that ubiquitin was not strongly immobilized or otherwise affected by the polymerization. Good quality spectra could be obtained on such a sample of ubiquitin at 4% gel with 4 mg/ml embedded and oriented purple membrane fragments (Table 1, ubi. 8). The residual orientation of ubiquitin in this sample yields residual $^1D_{NH}$ splittings in the range of ± 6 Hz (Figure 3C). A comparison of the orientation tensors before (Table 1, ubi. 7) and after the polymerization indicates very similar amplitudes and a good collinearity ($r = 0.97$, Table 2). As expected from earlier work on not immobilized purple membrane suspensions (Koenig et al., 1999; Sass et al., 1999), the orientation by the electrostatic interaction with the highly negatively charged purple membranes is very different from the steric orientation by uncharged lipid bicelles or by the strained gels. This is evidenced by the correlation coefficients of the orientation tensors in Table 2, which are approximately -0.3 with regard to the bicelles or ± 0.6 with regard to the strained gel systems.

Obviously, the method of residual orientation by the embedding of magnetically aligned substances into gels is not restricted to purple membranes, but can also be used with other systems as long as they are sufficiently inert to the polymerization process. Recent evidence (I. Kilpelainen, A. Annila, personal communication) suggests that similar alignments can be achieved by the embedding of magnetically aligned phages and viruses (Clore et al., 1998; Hansen et al., 1998). Furthermore, the method is also not restricted to a magnetic alignment of the embedded substance. Any other external macroscopic force, e.g. as a result of electric or mechanical interactions, can be used. Such orientations can be performed outside of the magnet. Taken together, these advantages open the way to a large variety of other procedures and substances which can be considered to obtain weak alignment.

Conclusions

In summary, we have shown that high quality spectra of non-trivial biomacromolecules can be obtained within the aqueous phase of polyacrylamide gels. For gels of 4–10% density, the concomitant increase in the rotational diffusion time of the solutes is much smaller than the reduction in their translational diffusion constant. Persistent weak alignments of solute macromolecules can be achieved in such a phase either by external macroscopic forces such as mechanical pressure or by the embedding of suitably oriented substances. The latter example of orientation by the embedding of purple membranes also illustrates the general possibility to study chemical or biological interactions of solute macromolecules with gel-immobilized substances. We are currently exploring the feasibility of such an approach.

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References

Arold, S., Franken, P., Strub, M.P., Hoh, F., Benichou, S., Benarous, R. and Dumas, C. (1997) *Structure*, **5**, 1361–1372.

Clore, G.M., Starich, M.R. and Gronenborn, A.M. (1998) *J. Am. Chem. Soc.*, **120**, 10571–10572.

Cordier, F., Dingley, A.J. and Grzesiek, S. (1999) *J. Biomol. NMR*, **13**, 175–180.

Cornilescu, G., Marquardt, J.L., Ottiger, M. and Bax, A. (1998) *J. Am. Chem. Soc.*, **120**, 6836–6837.

Dingley, A.J., Mackay, J.P., Chapman, B.E., Morris, M.B., Kuchel, P.W., Hambly, B.D. and King, G.F. (1995) *J. Biomol. NMR*, **6**, 321–328.

Dresselhaus, D. (1988) Ph.D. Thesis, Technische Universität Berlin.

Grzesiek, S., Bax, A., Hu, J.S., Kaufman, J., Palmer, I., Stahl, S.J., Tjandra, N. and Wingfield, P.T. (1997) *Protein Sci.*, **6**, 1248–1263.

Hansen, M.R., Rance, M. and Pardi, A. (1998) *J. Am. Chem. Soc.*, **120**, 11210–11211.

Kay, L.E., Nicholson, L.K., Delaglio, F., Bax, A. and Torchia, D.A. (1992) *J. Magn. Reson.*, **97**, 359–375.

Koenig, B.W., Hu, J.-S., Ottiger, M., Bose, S., Hendler, R.W. and Bax, A. (1999) *J. Am. Chem. Soc.*, **121**, 1385–1386.

Lewis, B.A., Rosenblatt, C., Griffin, R.G., Courtemanche, J. and Herzfeld, J. (1985) *Biophys. J.*, **47**, 143–150.

Sass, J., Cordier, F., Hoffmann, A., Cousin, A., Omichinski, J.G., Lowen, H. and Grzesiek, S. (1999) *J. Am. Chem. Soc.*, **121**, 2047–2055.

Tjandra, N. and Bax, A. (1997) *Science*, **278**, 1111–1114.

Tjandra, N., Feller, S.E., Pastor, R.W. and Bax, A. (1995) *J. Am. Chem. Soc.*, **117**, 12562–12566.

Tolman, J.R., Flanagan, J.M., Kennedy, M.A. and Prestegard, J.H. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 9279–9283.

Tycko, R., Blanco, F.J. and Ishii, Y. (2000) *J. Am. Chem. Soc.*, **122**, 9340–9341.

Vijay-Kumar, S., Bugg, C.E. and Cook, W.J. (1987) *J. Mol. Biol.*, **194**, 531–544.

Warrass, R., Wieruszeski, J.-M., Boutillon, C. and Lippens, G. (2000) *J. Am. Chem. Soc.*, **122**, 1789–1795.