6. Conclusion and general remarks

In the past few years NMR spectroscopy has become an established technique in structural biology and every year a large number of new structures of biological macromolecules are determined at atomic resolution using NMR [268]. In addition to structural information NMR can deliver data which allow the characterization of dynamic properties of biological macromolecules. However, detailed investigations using the complete 3D structure of proteins are limited to the molecular weight range up to 30 kDa using ^{13}C and ^{15}N labelling and possibly up to 50 kDa using in addition deuteration of the molecules. The intention of this presentation was to introduce the basic techniques and conventions used in modern high resolution NMR applied to the study of biological macromolecules in aqueous solution. For the sake of limiting the review to a realistic length several topics of interest have been neglected. The discussion of the basic components concentrated on their application in experiments required for the sequential assignment and structure determination and much less on experimental techniques used in investigations of dynamic aspects of molecules in solution. In addition the discussion of relaxation processes concentrated on dipole-dipole interactions which are usually the dominant relaxation processes in biological macromolecules. With the ever higher magnetic fields strengths used, relaxation due to chemical shift anisotropy (CSA) gains importance since it increases with the square of the magnetic field. For example magnetization transfer through carbonyl carbons becomes less efficient at high magnetic fields because of the large CSA of these carbons. On the other hand CSA can be used in transverse relaxation-attenuated (TROSY) experiments [269] at high magnetic fields to reduce transverse relaxation by a constructive use of the interference between dipole-dipole coupling and CSA. TROSY experiments have the potential to make possible detailed NMR studies of proteins much larger than 50 kDa.

In this review the author hopes to have made clear that NMR of biological macromolecules is extremely rich in experimental techniques which provide a flexibility of the method to adapt to peculiarities of the system investigated. This article is intended to introduce the newcomer to the field to technical and methodological aspects of NMR with proteins in solution from basic concepts to the modern implementations and applications using hydration studies as an example. To reach this goal, a building block approach was chosen starting with the discussion of simple elements such as rf pulses or gradients, proceeding to simple key segments and finally presenting some combinations of key segments. Along with the discussion of these elements and basic segments experimental details were introduced which are essential for a successful application of NMR with biological macromolecules. This outline should help the reader to find special topics again later for further reference.

Acknowledgements

Appendix

A.1. The Bloch equations

In the framework of classical physics the nuclear spins in an external magnetic field B_o create a magnetization M along B_o. NMR is described classically by the precession of this magnetization vector $\underline{M}$ about externally applied magnetic fields. The equations of motion for the magnetization vector $\underline{M} = (M_x, M_y, M_z)$ under the action of the magnetic field vector $\underline{B} = (B_x, B_y, B_z)$ are known as the Bloch equations [30] and take the following form in the rotating frame

$$dM_x/dt = \gamma(M_y B_z - M_z B_y) - M_x/T_2$$
$$dM_y/dt = \gamma(M_z B_x - M_x B_z) - M_y/T_2 \qquad \text{(A.1.1)}$$
$$dM_z/dt = \gamma(M_x B_y - M_y B_x) - (M_z - M_o)/T_1$$

The components B_x and B_y are the components of the *rf* field $\underline{B}_1$ applied perpendicular to the z axis. The residual field B_z along the z axis depends on the frequency difference between a particular resonance and the rotation frequency of the rotating frame. The vector sum of $\underline{B}_1$ and $\underline{B}_z$ results in an effective field $\underline{B}_{eff}$ with an amplitude B_{eff} and with an angle Θ to the z axis

$$B_z = B_o + \omega_{rf}/\gamma \qquad \text{(A.1.2)}$$

$$B_{eff} = (B_1{}^2 + B_z{}^2)^{1/2} \qquad \text{(A.1.3)}$$

$$\Theta = \arctan(B_1/B_z) \qquad \text{(A.1.4)}$$

The magnetization vector $\underline{M}$ precesses about the effective field $\underline{B}_{eff}$ in the rotating frame. With no B_1 field present the transverse magnetization in the rotating frame precesses about the residual field $\underline{B}_z$ with the frequency

$$\underline{\Omega} = -\gamma\underline{B}_z = -\gamma\underline{B}_o - \underline{\omega}_{rf} = \underline{\omega}_o - \underline{\omega}_{rf} \qquad \text{(A.1.5)}$$

where $\omega_o = -\gamma B_o$ is the Larmor frequency, the frequency which induces nuclear transitions. With $\omega_{rf} = -\gamma B_o$ the residual magnetic field B_z vanishes and magnetization components with this Larmor frequency remain stationary in this rotating frame unless a *rf* pulse is applied.

It is worth noting that the precession frequency ω_o of the magnetization is negative for nuclei with positive gyromagnetic ratios such as protons. The carrier frequency ω_{rf} has to be taken negative as well to obtain $\Omega = 0$. Setting the carrier frequency on the water resonance the precession of proton magnetization in the rotating frame is negative (from the x towards the $-y$ axis, in Fig. A1) for resonances in the spectrum left of the carrier and positive for those right of the carrier frequency [16, 31]. Thus in a ^{1}H spectrum of a protein with the frequency of the rotating frame set to the water resonance frequency, magnetization of aromatic protons precesses in the direction from the x towards the $-y$ axis and magnetization of methyl protons from the x towards the $+y$ axis.
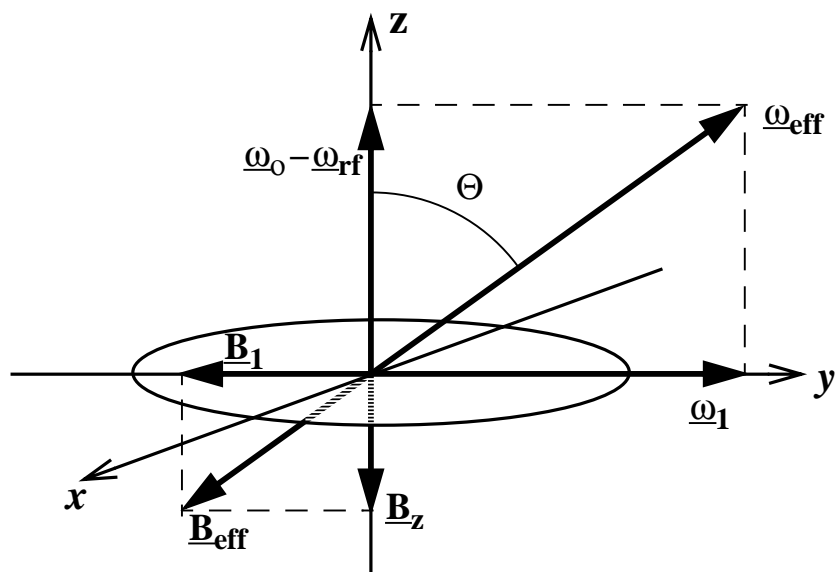
Fig. A1. Representation of the rotating frame where the z axis coincides with the external magnetic field B_o and which rotates with respect to the laboratory frame with the frequency ω_{rf} of the applied *rf* frequency pulses. Vectors are shown for the effective field $\underline{B}_{eff}$ which is the vector sum of the applied *rf* field $\underline{B}_1$ and the residual field $\underline{B}_z$. $\underline{B}_z$ represents the difference of the resonance frequency ω_o from ω_{rf}. Precession of magnetization components is governed by the corresponding angular frequency vectors with the values ω_{eff}, ω_1 and ($\omega_o - \omega_{rf}$) which are shown for a positive gyromagnetic ratio in the figure. Assuming B_1 to be on-resonance with the water resonance in a proton spectrum of a protein the magnetization corresponding to methyl protons precesses from the x towards the y axis after a 90° pulse applied along the y axis, magnetization corresponding to amide protons precesses from the x towards the $-y$ axis.

A.2. The product operator formalism

The product operator formalism applies only to spin systems with spins $\frac{1}{2}$ [28]. In addition it is assumed that there is no relaxation and that the chemical shift difference between two scalar coupled nuclei is much larger than their mutual scalar coupling J. In this framework the spin operators are transformed within their basis set (Table 2) by the three operators representing chemical shift, scalar coupling and *rf* pulses. The transformation of the spin operators during the course of a pulse sequence can be described by simple rules which are summarized in the following for the cartesian and the shift operator basis. The transformation under the individual operators is indicated by an arrow (--->).

Transformation under the chemical shift operator ($\omega\tau I_z$):

$$I_x \longrightarrow I_x \cos(\omega\tau) + I_y \sin(\omega\tau) \qquad I^+ \longrightarrow I^+ e^{-i\omega\tau}$$
$$I_y \longrightarrow I_y \cos(\omega\tau) - I_x \sin(\omega\tau) \qquad I^- \longrightarrow I^- e^{i\omega\tau} \qquad (A.2.1)$$
$$I_z \longrightarrow I_z \qquad\qquad\qquad\qquad I_z \longrightarrow I_z$$

Transformation under the scalar spin-spin coupling operator ($2\pi J\tau I_z S_z$):

$$I_x \quad \longrightarrow I_x \cos(\pi J\tau) + 2I_y S_z \sin(\pi J\tau) \qquad I^+ \quad \longrightarrow I^+ \cos(\pi J\tau) - 2iI^+ S_z \sin(\pi J\tau)$$
$$I_y \quad \longrightarrow I_y \cos(\pi J\tau) - 2I_x S_z \sin(\pi J\tau) \qquad I^- \quad \longrightarrow I^- \cos(\pi J\tau) + 2iI^- S_z \sin(\pi J\tau)$$
$$I_z \quad \longrightarrow I_z \qquad\qquad\qquad\qquad\qquad\qquad I_z \quad \longrightarrow I_z$$
$$2I_x S_z \longrightarrow 2I_x S_z \cos(\pi J\tau) + I_y \sin(\pi J\tau) \qquad 2I^+ S_z \longrightarrow 2I^+ S_z \cos(\pi J\tau) - iI^+ \sin(\pi J\tau) \qquad (A.2.2)$$
$$2I_y S_z \longrightarrow 2I_y S_z \cos(\pi J\tau) - I_x \sin(\pi J\tau) \qquad 2I^- S_z \longrightarrow 2I^- S_z \cos(\pi J\tau) + iI^- \sin(\pi J\tau)$$
$$2I_x S_x \longrightarrow 2I_x S_x \qquad\qquad\qquad\qquad\qquad 2I^+ S^+ \longrightarrow 2I^+ S^+$$
$$2I_y S_x \longrightarrow 2I_y S_x \qquad\qquad\qquad\qquad\qquad 2I^- S^+ \longrightarrow 2I^- S^+$$
$$2I_z S_z \longrightarrow 2I_z S_z \qquad\qquad\qquad\qquad\qquad 2I_z S_z \longrightarrow 2I_z S_z$$

Transformation of the cartesian spin operators under the operator for a *rf* pulse with angle β and phase ϕ (βI_ϕ):

$$I_x \longrightarrow -I_z \sin\beta \sin\phi + I_x (\cos\beta \sin^2\phi + \cos^2\phi) + I_y \sin^2(\beta/2) \sin2\phi$$
$$I_y \longrightarrow \ I_z \sin\beta \cos\phi + I_y (\cos\beta \cos^2\phi + \sin^2\phi) + I_x \sin^2(\beta/2) \sin2\phi \qquad (A.2.3)$$
$$I_z \longrightarrow \ I_z \cos\beta + I_x \sin\beta \sin\phi - I_y \sin\beta \cos\phi$$

Transformation of the shift spin operators under the operator for a *rf* pulse with angle β and phase ϕ (βI_ϕ):

$$I^+ \longrightarrow \ I^- \sin^2(\beta/2)\, e^{i2\phi} + I^+ \cos^2(\beta/2) + iI_z \sin(\beta)\, e^{i\phi}$$
$$I^- \longrightarrow \ I^- \cos^2(\beta/2) + I^+ \sin^2(\beta/2)\, e^{-i2\phi} - iI_z \sin(\beta)\, e^{-i\phi} \qquad (A.2.4)$$
$$I_z \longrightarrow -iI^- \sin(\beta)\, e^{i\phi}/2 + iI^+ \sin(\beta)\, e^{-i\phi}/2 + I_z \cos(\beta)$$

For the most frequently used *rf* pulses with flip angle 90° and 180° and with *rf* phases which are multiples n of $\pi/2$ (90°) the transformation rules become much simpler and can be written in a compact form. For 180° pulses the following rules are obtained

$$I_x \longrightarrow (-1)^n I_x \qquad\qquad\qquad\qquad I^+ \longrightarrow (-1)^n I^- \qquad\qquad (A.2.5)$$
$$I_y \longrightarrow (-1)^{n+1} I_y \qquad\qquad\qquad\qquad I^- \longrightarrow (-1)^n I^+$$

and for 90° pulses

$$I_x \longrightarrow - I_z \sin(n\pi/2) + I_x \cos^2(n\pi/2) \qquad I^+ \longrightarrow (-1)^n I^- / 2 + I^+ / 2 + i^{n+1} I_z$$
$$I_y \longrightarrow I_z \cos(n\pi/2) + I_y \sin^2(n\pi/2) \qquad I^- \longrightarrow I^- / 2 + (-1)^n I^+ / 2 + (-i)^{n+1} I_z \qquad (A.2.6)$$
$$I_z \longrightarrow I_x \sin(n\pi/2) - I_y \cos(n\pi/2) \qquad I_z \longrightarrow -(i)^{n+1} I^- / 2 - (-i)^{n+1} I^+ / 2$$

Operators transform individually under the rules formulated in Eqs. A.2.1–A.2.6 even in products of operators, except for anti-phase terms (Table 2) which have to be treated as an unit using Eq. A.2.2. However, these terms can be treated consecutively when different couplings to the same spin exist.

Even though calculations with the product operator formalism are in principle easy, quite a large number of terms may have to be treated when describing a pulse sequence making computer programs performing the calculations very attractive [36, 37].