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Double quantum sodium NMR studies of the halotolerant Ba_1 bacterium

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Abstract

The relaxation times T_1, T_{2f} and T_{2s} of sodium in the cytosol of the halophilic halotolerant bacterium Ba_1 isolated from the Dead Sea were measured. T_{2f} and T_{2s} were separated by using the double quantum filtering method. The measurements were carried out at two different concentrations of the growth medium. From the spectral density ratio it seems that the microenvironment in the cytosol affecting the sodium relaxation times is similar for the two different growth media. It seems that the bacteria keep a constant microenvironment in the cytosol.

Keywords: Sodium NMR; Double quantum filtering; Halobacteria, Ba_1; Relaxation times

1. Introduction

Eubacterial halophiles are able to accommodate their growth and metabolism to wide variation in the external salinity of the growth medium [1–3]. However, over a very wide concentration range of sodium in the medium, the concentration of the intracellular sodium was lower than that in the growth medium [4,5]. The intracellular osmotic pressure was maintained by various compatible solutes, such as potassium, glycine–betaine, etc. [6–9]. Parameters which might indicate changes in the intracellular microenvironment in which the Na is embedded are the sodium NMR relaxation times T_1 and T_2 [10–13].

The sodium-23 nucleus has a spin quantum number $I = 3/2$. This causes the relaxation of the longitudinal and transverse magnetisation to be biexponential [10,11,14,15]. The relaxation times T_{2s} and T_{2f} are the slow and fast components of the transverse relaxation. They can be deduced from the lineshape of the spectrum [16], or from the Carr–Purcell–Meiboom–Gil (CPMG) pulse sequence [17]. In many cases, the broad component of the line that represents the fast relaxation T_{2f} is embedded in the noise and difficult to detect unless a very good signal-to-noise ratio is obtained. Recently, the use of multiple quantum filtering (MQF) to obtain T_{2s} and T_{2f} was proposed [18,19] and used on several biological and model systems [20–26]. From the relaxation times, information about the sodium interaction with macromolecules and other binding sites can be deduced [12,13].

In this study we employ the double quantum filtering (DQF) techniques for the study of T_{2s} and T_{2f} of the sodium in the cytosol of Ba_1

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bacteria at several sodium concentrations in the growth medium. Together with T_1 measurement we gain information about the intracellular environment in which the sodium is embedded.

2. Background

The sodium nucleus has a spin $I = 3/2$ and hence a nuclear quadrupole moment. This feature complicates the NMR spectrum as compared to nuclei that have $I = 1/2$, such as ^1H, ^{13}C or ^{31}P. Another complication arises from the fact that in aqueous solutions the sodium appears as Na^+ ions, and there is no chemical shift between sodium ions in different aqueous environments. In a system occluded by a membrane from the environment, and where large ions cannot penetrate the membrane, it is possible to differentiate between the inner and the outer environment by using a reagent that shifts the sodium from one solution to the other and thus separating the sodium signals [27,28]. This method was applied for the determination of sodium properties in cells and tissues [5,25,29].

The pattern of the sodium NMR spectrum is governed by the special properties of the spin $I = 3/2$. There are two spin–lattice relaxation times — a slow and a fast one — T_{2s} and T_{2f} [10,11,14,15]. Since the NMR line shape depends on T_2 values, when T_{2s} and T_{2f} are different the line shape is a superposition of two Lorentzians. When the T_{2f} is much shorter than T_{2s} the broad line connected with T_{2f} may disappear because of experimental imperfections [10,30]. It was realized that for a system of $I = 3/2$ where $T_{2s} > T_{2f}$ the multiple quantum coherence (MQC) does not vanish [13,14]. MQC is measured by the pulse sequence [18] $90° - \tau/2 - 180° - \tau/2 - 90° - t - 90°$ aq, called multiple quantum filtering (MQF) and its line shape after Fourier transformation is given by eq. (1), which is given for the case of double quantum filtering (DQF).

$$g(\omega) = K(e^{-\tau/T_{2f}} - e^{-\tau/T_{2s}}) \times e^{-t/T_{2DQ}}\left(\frac{T_{2s}}{1+\Delta\omega^2T_{2s}^2} - \frac{T_{2f}}{1+\Delta\omega^2T_{2f}^2}\right) \quad (1)$$

The line shape is composed of a difference between two Lorentzians and it depends on the delay times between the pulses τ and t. On measuring the DQC as a function of τ, the relaxation times T_{2f} and T_{2s} can be determined.

For spin $I = 3/2$, the relaxation times are given by the following equations [10,25]

$$\frac{1}{T_{1f}} = 2C^2J_1(\omega_0) \quad (2)$$

$$\frac{1}{T_{1s}} = 2C^2J_2(2\omega_0) \quad (3)$$

$$\frac{1}{T_{2f}} = C^2\big(J_0(0) + J_1(\omega_0)\big) \quad (4)$$

$$\frac{1}{T_{2s}} = C^2\big(J_1(\omega_0) + J_2(2\omega_0)\big) \quad (5)$$

$$\frac{1}{T_{2DQ}} = C^2\big(J_0(0) + J_2(2\omega_0)\big) \quad (6)$$

where the spectral densities $J(\omega)$ are given by

$$J_n(n\omega_0) = \frac{\tau_c}{1+n^2\omega_0^2\tau_c^2} \quad (7)$$

and C represents the quadrupole interaction in an axially symmetrical field.

$$C^2 = \frac{1}{20}\left(\frac{e^2Qq}{\hbar}\right)^2 \quad (8)$$

The correlation time τ_c and through it $J_n(\omega)$ may give some insight on the microenvironment in which the nucleus is embedded. Short τ_c values mean fast molecular motions that affect the relaxation of the nuclear spin while long τ_c values indicate restricted motions. For short τ_c values where $\omega^2\tau_c^2 \ll 1$ the spectral densities $J_0(0) = J_1(\omega_0) = J_2(2\omega_0)$ but for long τ_c values they are different. So through the measurements of the relaxation times and the calculation of the spectral density information about the molecular motions affecting the sodium nucleus may be obtained.

3. Experimental

All materials were of analytical grade and purchased from commercial sources except sodium triphosphate (Alpha Products) that was recrystallized three times from water–ethanol mixtures [31].

The Ba_1 bacteria were isolated from the evaporation pools near the Dead Sea by D. Rafaeli-Eshkol [32]. The Ba_1 bacteria were grown at 37°C under vigorous shaking. The growth medium contained 0.8% (w/v) nutrient broth (Difco) 50 m*M* $MgCl_2$ and 0.8 or 0.4 *M* NaCl. The cells were harvested at the late exponential phase of growth and washed in the same medium but without nutrient broth. The pellet (300–400 mg wet wt.). was resuspended in 2 ml of NaCl medium that contained dysprosium bis(tripolyphosphate) ion ($Dy(TPP)_2^{7-}$) which served as the shift reagent (added as the sodium salt) between the sodium in the medium and that in the cytosol.

3.1 NMR measurements

Sodium NMR studies were carried out on a Bruker AM400WB at a frequency of 105 MHz, T_1 values were determined by the $180° - \tau - 90°$ pulse sequence. Double quantum filtering was measured by the $90° - \tau/2 - 180° - \tau/2 - 90° - t - 90°$ aq; pulse sequence. The phase cycle was given by Bruker and was similar to that used by Seo et al. [20,22]. T_{2s} and T_{2f} were determined from eq. (1) by varying the value of τ. T_{2DQ} was determined from the exponential decay obtained when t was varied. The NMR measurements were carried out on resting cells at ambient temperature of ~ 24°C. The time of each experiment was several hours. To ensure the reliability of the results the conditions of the first point were repeated at the end of the experiment.

4. Results and discussion

The shift reagent $Dy(TPP)_2^{7-}$ that was introduced to the outer solution of the Ba_1 bacteria effected a shift between the intracellular and extracellular sodium. The solubility of $Na_7Dy(TPP)_2$ is limited and hence the sodium shift at high sodium concentrations is restricted [5]. To get good separation between the two signals we worked at a maximum concentration of 0.8 *M* NaCl in the growth medium. In Fig. 1a a spectrum of one-quantum coherence spectrum is presented, the separation between the sodium in the two environments is observed. In Fig. 1b the double-quantum coherence version of the same spectrum is presented. It can be seen from Fig. 1a that the ratio of the extracellular sodium to the intracellular sodium is very high, and the small inner sodium signal overlaps the shoulder of the much stronger outer signal. The extracellular sodium does not behave as free sodium, in the thick suspension of cells there is a broadening of the linewidth of the sodium detected (Fig. 1a). As a result there is also a difference between T_{2f} and T_{2s} and hence a detectable double quantum coherence (DQC) spectrum for the intracellular as

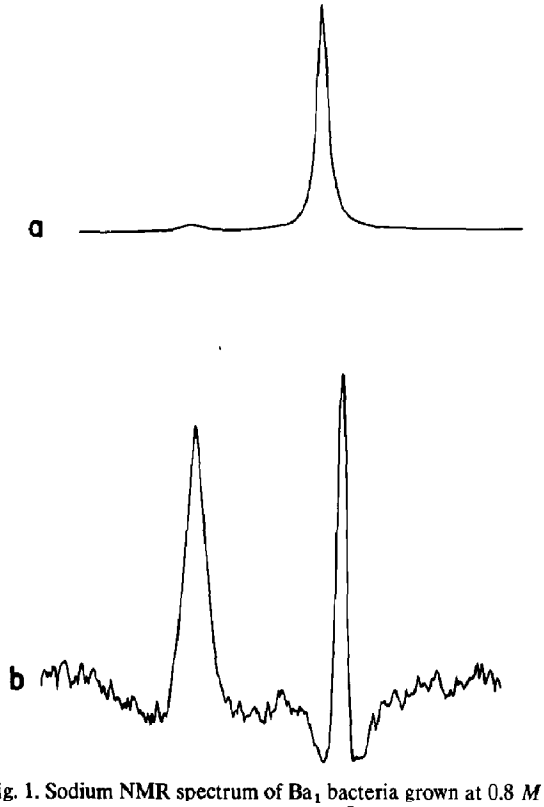

Fig. 1. Sodium NMR spectrum of Ba_1 bacteria grown at 0.8 *M* NaCl by using shift reagent $Dy(TPP)_2^{-7}$ (a) Single quantum coherence spectrum, and (b) double quantum coherence spectrum.

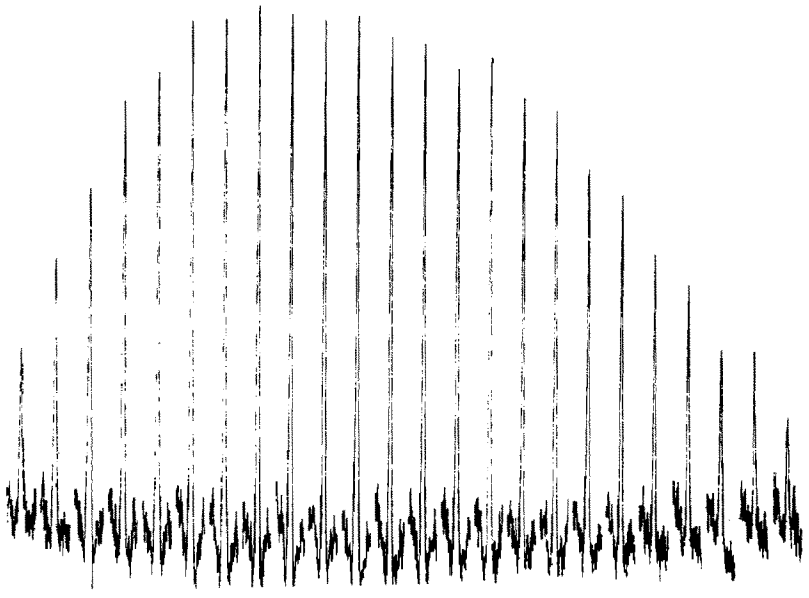

Fig. 2. The double quantum coherence signal of intracellular sodium as a function of the time.

well as for the extracellular sodium (Fig. 1b). In Fig. 2 the DQC spectrum of the intracellular sodium at different τ values is presented. The general behaviour of the line intensity fits eq. (1). Figure 3 gives the simulation of the experimental results to eq. (1), together with the experimental points derived from Fig. 2. By this simulation the values of T_{2f} and T_{2s} are determined from the fit. On changing the time t in the pulse sequence the value of T_{2DQ} can be derived from eq. (1). Figure 4 gives the DQC spectrum at different t values.

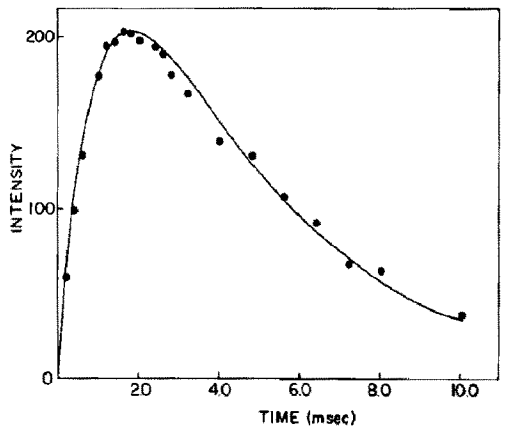

Fig. 3. Double quantum coherence intensity as a function of τ; (●) experimental results, (—) calculated from eq. (1).

The results of T_1, T_{2f}, T_{2s} and T_{2DQ} are summarized in Table 1 for two concentrations of the growth medium, 0.4 M NaCl and 0.8 M NaCl, respectively.

The results presented in the table show that $T_1 \gtrsim T_{2s} \gg T_{2DQ} \gtrsim T_{2f}$ at the two growth media. The above results demonstrate that the correlation times for the sodium molecular motions are in the region where τ_c is long ($\omega_0\tau_c \gtrsim 1$). It was not possible to calculate the τ_c values from the experimental results presented here, but we calculated the ratios of the spectral densities from eqs. (4) to (8). The calculations give a rough ratio of $J_0(0) \dot{-} J_1(\omega) \dot{-} J_2(2\omega) = 1 \dot{-} 0.25 \dot{-} 0.15$ for the 0.4 M NaCl medium and $J_1(0) \dot{-} J_1(\omega) \dot{-} J_2(2\omega) = 1 \dot{-} 0.35 \dot{-} 0.1$ for 0.8 M NaCl in the medium.

Table 1

The sodium intracellular relaxation times at various NaCl concentration in the growth medium

medium concentration NaCl [M]	T_1 (ms)	T_{2S} (ms)	T_{2F} (ms)	T_{2DQ} (ms)
0.4	6.9±1.1	4.6±1	0.35±0.09	0.49±0.06
0.8	8.6±1.2	3.5±0.65	0.6 ±0.2	0.87±0.19

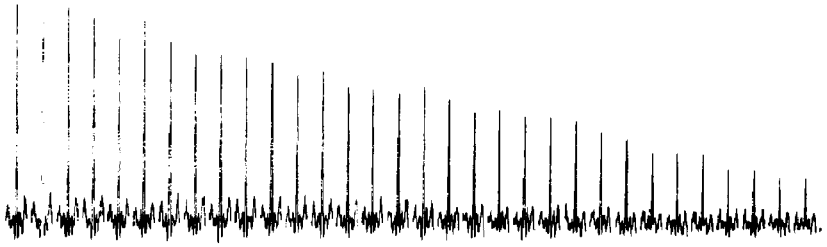

Fig. 4. The double quantum coherence signal of the intracellular sodium as a function of the time t.

From the results we may conclude that τ_c is long, since $\omega_0 = 105$ MHz the value for τ_c can be estimated to be $\tau_c \geq 10^{-8}$ s compared to 10^{-11}–12^{-12} s for free sodium. It seems that there is not much difference between the values of the relaxation times in the two media, and the ratios of the spectral densities do not differ as well. It may be concluded that the Ba_1 bacterium keeps a constant microenvironment in the cytosol.

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