PMR STUDY OF tRNA COMPLEXES WITH LOW-MOLECULAR-WEIGHT COMPOUNDS USING Mn^{2+} ions

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Received 13 November 1972

1. Introduction

PMR studies of complexes of biopolymers with low-molecular-weight compounds are hampered by the comparatively small differences between the proton spectral parameters for the complex and the free state. This difficulty can be overcome by the addition of paramagnetic ions which, binding with the biopolymer, cause sharp changes in the spectral characteristics of the protons of the low-molecular-weight compounds by paramagnetic broadening and shifts of resonance lines.

Accordingly, we have undertaken a study of the complexing of tRNA with the organic cation tetramethylammonium (TMA) in the presence of Mn^{2+} ions, known for their strong affinity for tRNA (cf. [1, 2]).

It had already been shown [3] that TMA formed complexes with tRNA phosphates.

2. Experimental

Unfractionated yeast tRNA (Institute of Organic Chemistry, Novosibirsk, USSR) and TMA hydrochloride were used. Spectra were recorded on the JNM-4H-100 spectrometer in D_2O using tert-butanol as internal reference.

3. Results and discussion

Fig. 1. shows a plot of the linewidths of the water and TMA protons vs. tRNA concentration for constant concentrations of TMA and Mn^{2+}. Perceptible line broadening sets in at a nucleotide concentration of 10^{-3} M. In the absence of the Mn^{2+} ions, broadening of the TMA signal was only observed to begin with 0.1 M tRNA [3]. Clearly, the drastic distinction in the paramagnetic broadening of TMA signal in the tRNA complex must be due to the dipole–dipole coupling of the protons with complexed Mn^{2+} ions. It was therefore assumed that maximum broadening of the TMA proton signals would occur at complete binding of the Mn^{2+} ions.

Indeed, fig. 1 shows that the TMA and water proton linewidths are constant, when the concentration ratio of Mn^{2+} and tRNA ([Mn]/[P]) is below 0.15. At such ratios the Mn^{2+} ions are strongly bound to tRNA [4–6].

In order to determine the linewidth of TMA complexed with tRNA and the stability constant of this complex, the measured TMA proton linewidths were plotted against the TMA concentration, all Mn^{2+} ions being bound. The plot of $1/(\Delta\nu - \Delta\nu_f)$ vs. [TMA] is presented in fig. 2. From its linear character the stability constant of TMA–tRNA complex can be calculated from eq. (1)

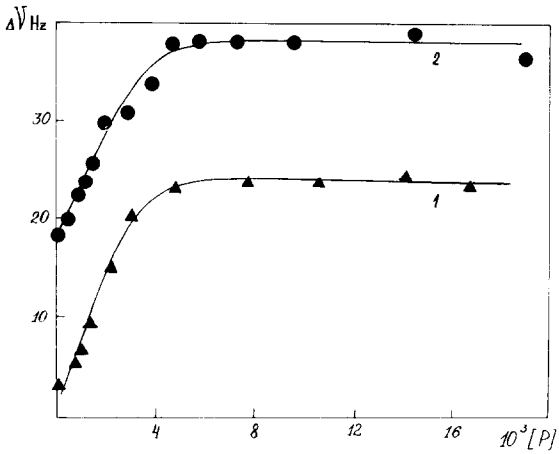

Fig. 1. TMA-proton (1) and H_2O proton (2) linewidths vs. tRNA concentration. The TMA·HCl (0.062 M) and $MnSO_4$ (5.6×10^{-4} M) concentrations were constant.

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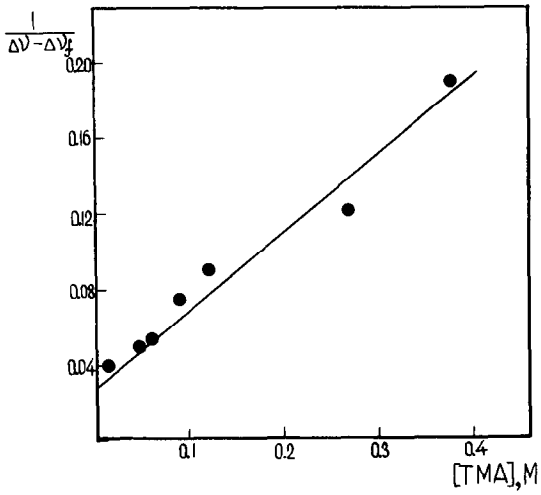

Fig. 2. Inverse TMA proton linewidths $1/(\Delta\nu-\Delta\nu_f)$ vs. TMA concentration. The $MnSO_4$ (5×10^{-4} M) and tRNA (1.2×10^{-2} M) concentrations were constant.

$$K = \frac{[TMA]_b}{\{[Mn]_0 - [TMA]_b\}[TMA]_f} \approx \frac{[TMA]_b}{\{[Mn]_0 - [TMA]_b\}[TMA]_0} \quad (1)$$

where the subscripts f, b designate the concentration of TMA in the free and bound state, respectively; 0. the total TMA concentration and $[Mn]_0$ the concentration of complexed Mn^{2+} ions, which may be called the concentration of the "broadening" sites in tRNA. Since the observed TMA proton linewidth observed is:

$$\Delta\nu = \Delta\nu_b \frac{[TMA]_b}{[TMA]_0} + \Delta\nu_f \frac{[TMA]_f}{[TMA]_0} \quad (2)$$

where $\Delta\nu_b$ is its width in the tRNA complex and $\Delta\nu_f$, its width in the absence of tRNA, the linear dependence

$$\frac{1}{\Delta\nu - \Delta\nu_f} = \frac{[TMA]_0}{\Delta\nu_b \cdot [Mn]_0} + \frac{1}{K \cdot \Delta\nu_b \cdot [Mn]_0} \quad (3)$$

shown in fig. 2 can be obtained.

It can be seen from (3) that the slope and the intersection of the line in fig. 2 with the ordinate, are determined by the values of K and $\Delta\nu_b$ these being $K = 13.0$ M^{-1}, $\Delta\nu_b = 5.0 \times 10^3$ Hz. The K value is in good agreement with the results of studies of tRNA complexes with other organic cations [7]. At the same time the TMA proton linewidth in the complex is considerably greater than the maximum linewidth 8–30 Hz obtained for organic cations and zwitterions [3, 7]. Such a high value of $\Delta\nu_b = 5.0 \times 10^3$ Hz makes it possible to determine the stability constant of the tRNA–TMA complex, when less than 1% of TMA molecules are complexed. According to the Solomon–Bloembergen theory (see, e.g. [8]) the paramagnetic dipole–dipole broadening of complexed protons is given by:

$$\Delta\nu_b = A \cdot q \frac{\tau_c}{r^6} \quad (4)$$

where A is a theoretically calculated constant, τ_c the dipole–dipole correlation time; r the distance between ion and proton; and q the coordination number, which is assumed to be 1. It has been shown [9] that at 30°, for a water proton in the sphere of a Mn^{2+} ion coordinated by tRNA, $\tau_c = 2.5 \times 10^{-9}$ sec. We have used these data [9] to determine the mean distance between the Mn^{2+} ions and TMA protons, which proved to be 7.3 Å. The value obtained indicates that the TMA molecules adjacent to the coordinated ion are located at the neighbouring phosphate.

It should be noted that paramagnetic ions may also be used to investigate complex formation of biopolymers with inorganic cations. Towards this end it is convenient to make use of the competition between organic and inorganic cations for the biopolymer binding sites. For instance, in fig. 3 the plot of TMA linewidth vs. Na^+ ion concentration is presented for constant TMA, tRNA and Mn^{2+} concentrations. This de-

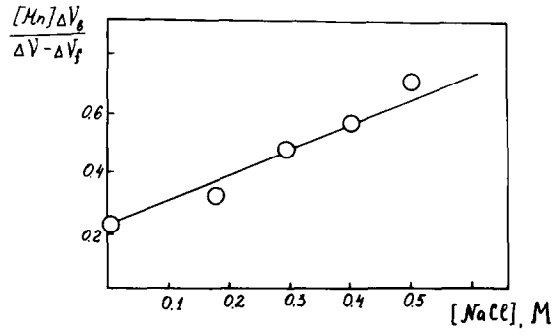

Fig. 3. Plot of $[Mn] \cdot \Delta\nu_b/(\Delta\nu-\Delta\nu_f)$ vs. [NaCl]. The TMA·HCl (0.14 M), $MnSO_4$ (5×10^{-4} M) and tRNA (1.2×10^{-2} M) concentrations were constant.

pendence can be easily described by:

$$\frac{[Mn]\cdot\Delta\upsilon_b}{\Delta\upsilon-\Delta\upsilon_f}=\frac{1}{K_{TMA}}+[TMA]+\frac{K_{Na}}{K_{TMA}}[Na] \qquad (5)$$

where K_{Na} is the stability constant of Na^+ ions with tRNA phosphate groups. The value of the constant found from these data using (5) was 10 M^{-1}.

In conclusion it should be emphasized that the evidence about tRNA complexes was obtained for comparatively low tRNA concentrations. Under such conditions the stability constants and bound ion–low-molecular-weight compound distances can be found with the aid of Mn^{2+} ions. Taking into account the variety of biopolymer complexes with paramagnetic ions this technique is believed to be of general interest.

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