A SPIN-LABEL FOR RNA STUDY

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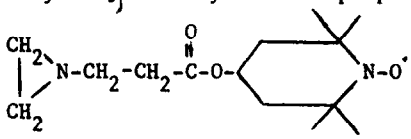
Abstract

The compound 2,2,6,6-tetramethyl-4-[β-N-ethyleneiminopropionyl] oxypiperidine-I-oxyl is used as a spin-label for RNA. The reaction, effected under rather mild conditions, results in 50-70 nucleotides per spin-label. The temperature dependence of the ESR spectra of spin-labeled RNA is used to estimate temperatures corresponding to the beginning of melting, T_{crit} ("critical" points of the structure) and to calculate the effective activation energies of the rotational mobility of spin-labels, $\Delta E_{eff.}$; the dependence of $T_{crit.}$ on the ionic strength of the solution is also determined.

The spin-label approach[1,2] to the study of nucleic acids, as suggested by Smith and Yamane[3], was previously limited essentially by the absence of suitable effective spin-labels. Recently we succeeded in substantiating that 2,2,6,6-tetramethyl-4-[β-N-ethyleneiminopropionyl] oxypiperidine-I-oxyl



I

may be used to obtain spin-labeled DNA preparations[4]. We now report on the use of this spin-label for studying the conformational change in RNA.

Materials and Methods

Compound I is synthesised according to method[5]. A repeatedly purified preparation of I6S RNA is isolated from cells of B. subtilis. The addition reaction of the spin-label to RNA is curried out in 0.I M acetate buffer, pH 5.5, at room temperature for three days. The unreacted radical is removed either by intensive dialysis (one day) or repeated RNA precipitation in ethanol. The resulting RNA contains no less than 50-70 polymer bases per spin-label. It is to be noted that spectra of circular dichroism and those of UV absorption for preparations of native and spin-labeled RNA are found to be similar. ESR spectra are taken on the radiospectrometer ESR-2 with the accuracy of thermostating ±0.5°.

Results and Discussion

The ESR spectrum of the spin-labeled RNA preparation is shown in fig. I.

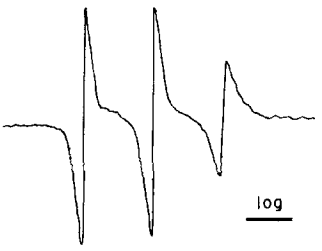


Fig. I. ESR cpectrum of RNA preparation labeled with compound I; 22°C, 0.I M NaCL, RNA 200 γ/ml.

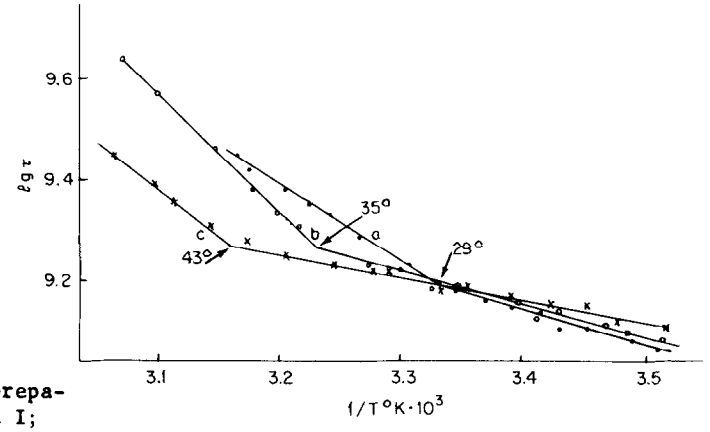


Fig. 2. The dependence of the correlation time of the spin-label on RNA upon temperature in terms of Arrhenius coordinates: a) 0.0I M NaCl, b) 0.I M NaCl, c) I.0 M NaCl.

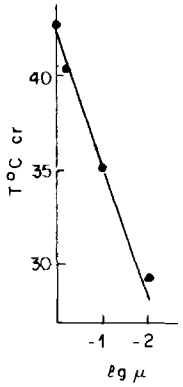


Fig. 3. Dependence of $T_{crit.}$ upon the logarithm of the ionic strength of the solution (NaCl).

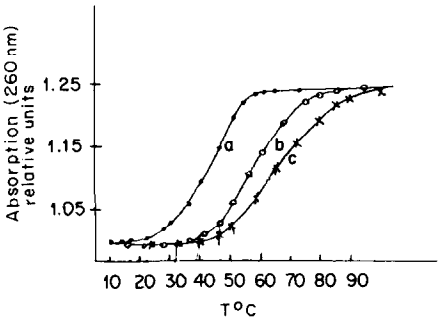


Fig. 4. Melting curves of RNA: a) 0.0I M NaCl, b) 0.I M NaCl, c) I.0 M NaCl.

The spectrum reveals a considerable limitation of the rotational mobility of the nitroxyl fragment of the label (the correlation time, τ , being $6.8 \cdot 10^{-10}$ sec., as calculated from[6]). The ESR spectra parameters remain unaffected (at room temperature) when the ionic strength of the solution is raised from 2 to 5 10^{-3} M NaCl or when RNA is diluted.

Fig. 2 shows the dependence of on T in terms of Arrhenius equation as:

$$\tau = \tau_0 \, e^{-\frac{\Delta E_{eff.}}{R\,T}} \qquad (I)$$

in solutions with various ionic strength. It will be readily seen that the curves have two linear sections whose intersection points correspond to

$T_{crit.}$. These temperatures may be correlated with the ionic strength of the solutions μ by equation (see fig.3):

$$T_{crit.} = a + b \lg\mu ; \quad (a=43°, b=-7°). \qquad (2)$$

It is difficult to determine unequivocally when RNA melting starts because in this region absorption at 260 nm is slurred (fig.4). On the other hand it is possible to register the "critical" state temperature of RNA structure by means of the spin-label ($T_{crit.} < T_{melt.}$), after which RNA appears to acquire a dynamically new, "metastable" state, preceding melting.

Table I gives parameters of the spin-labeled RNA melting in solutions with different ionic strength.

TABLE I

Some parameters characteristic of spin-labeled RNA in solutions with different ionic strength

ionic strength M NaCl	$T^{\circ}_{crit.}$	$T^{\circ}_{melt.}$	ΔE_{eff} kcal/mole $T < T_{crit.}$	ΔE_{eff} kcal/mole $T > T_{crit.}$
0.0I	29	43	I.42	3.06
0.I	35	56	I.27	4.50
I.0	43	66	I.0I	3.80

It is important to note that when $T < T_{crit.}$ the dynamic structure of RNA is changing rather slowly ($\Delta E_{eff.}$ from equation (I) being small) whereas when $T > T_{crit.}$ the rate of τ changing with temperature rises sharply (big $\Delta E_{eff.}$). Hence, as soon as T exceeds $T_{crit.}$ the temperature rise is accompanied by a rather fast change in RNA structure.

Compound I is thus an effective spin-lable providing information on conformational changes and dynamic RNA structure in solution. It is believed, that our $T_{crit.}$ which is rather concrete and unambiguous may prove useful for the study and theoretical description of the denaturation process of nucleic acids.

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