Multinuclear n.m.r. studies on sol–gel transition of κ-carrageenan. Selective interaction of group I cations

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Multinuclear n.m.r. experiments were carried out to study a selective interaction between group I cations and κ-carrageenan. It was found that the n.m.r. intensity of gel-forming cations undergoes a rapid change in the vicinity of sol–gel transition temperature. On the other hand, the n.m.r. intensity of a non-gel forming cation which coexists with a gel-forming cation does not show any singular behaviour at the sol–gel transition. These results strongly suggest that gel-forming cations selectively interact with κ-carrageenan.

(Keywords: multinuclear n.m.r.; sol–gel transition; κ-carrageenan; group I cation)

INTRODUCTION

The sol–gel transition of various polysaccharides has been of great interest since molecular models of the sol–gel transition have been proposed[1,2]. The sol–gel transition of κ-carrageenan is of particular interest because the gelation of κ-carrageenan is markedly enhanced by the presence of a group I cation in solution. Group I cations are divided into two classes: one contains gel-forming cations (K^+, Rb^+ and Cs^+) and the other contains non-gelling cations (Li^+ and Na^+). It has been known that κ-carrageenan forms a gel by Li and Na ions. The concentration of these ions necessary to form gel is, however, much higher than that of other group I cations; typically the concentration of about 1 M is necessary to form a gel by Li and Na ions. In this study, therefore, we regard these ions as 'non-gelling cations' because the concentration of Na ion is much less than 1 M in our studies[2,3].

The role of group I cations in gelation of κ-carrageenan solution and the motional state in the vicinity of the transition point are, however, not yet fully understood. It has been suggested by several workers that the observation of multinuclear magnetic resonance (n.m.r.) is useful in the study of the sol–gel transition of κ-carrageenan[3–7]. In this work we apply the multinuclear n.m.r. technique to study the selective interaction between group I cations and κ-carrageenan molecules. We hope to show a direct evidence of the selective interaction between group I cations and κ-carrageenan.

EXPERIMENTAL

κ-carrageenan from *Euchuma cottonii* was purchased from Sigma Chemical Co. The crude sample was purified by successive dialysis against 10 mM solution of EDTA and distilled water. It was lyophilized after neutralization by NaOH.

Sodium κ-carrageenan was dissolved in three different solvents at a temperature of 80°C in 15 mm n.m.r. tubes. Sample I contains 20 mM NaCl and 20 mM RbCl; sample II contains 20 mM NaCl and 20 mM CsCl; sample III contains 20 mM NaCl, 10 mM RbCl, and 10 mM CsCl. The concentration of κ-carrageenan was kept at 3.0 g/100 ml. Solutions were rapidly cooled to 0°C for 20 min to transform into gel. The n.m.r. spectrum was observed immediately after gelation. The composition of three samples studied here is given in *Table 1*.

N.m.r. measurements of ^{23}Na, ^{87}Rb and ^{133}Cs were made on a Jeol JNM-GX500 spectrometer operating at a field strength of 11 T. The temperature was changed from 25 to 60°C with an interval of 5°C. The spin–spin relaxation time T_2^* was calculated from the half line width of the spectrum $\nu_{1/2}$ using the following relationship[8].

$$T_2^* = 1/\pi\nu_{1/2}$$

The melting temperature of the gel was determined by the falling ball method using a Teflon ball of 1.6 mm in diameter and 5 mg in weight. The melting temperature thus obtained is also given in *Table 1*.

RESULTS

The temperature dependence of the n.m.r. spectrum of ^{23}Na, ^{87}Rb and ^{133}Cs in samples I, II and III is given in

Table 1 The concentration of group I cations and κ-carrageenan for three samples studied. The melting temperature of gels is given in the last column

Sample	NaCl (mM)	RbCl (mM)	CsCl (mM)	κ-Carrageenan	T_m (°C)
I	20	20	–		43.7
II	20	–	20	3.0 g/100 ml	34.2
III	20	10	10		38.9

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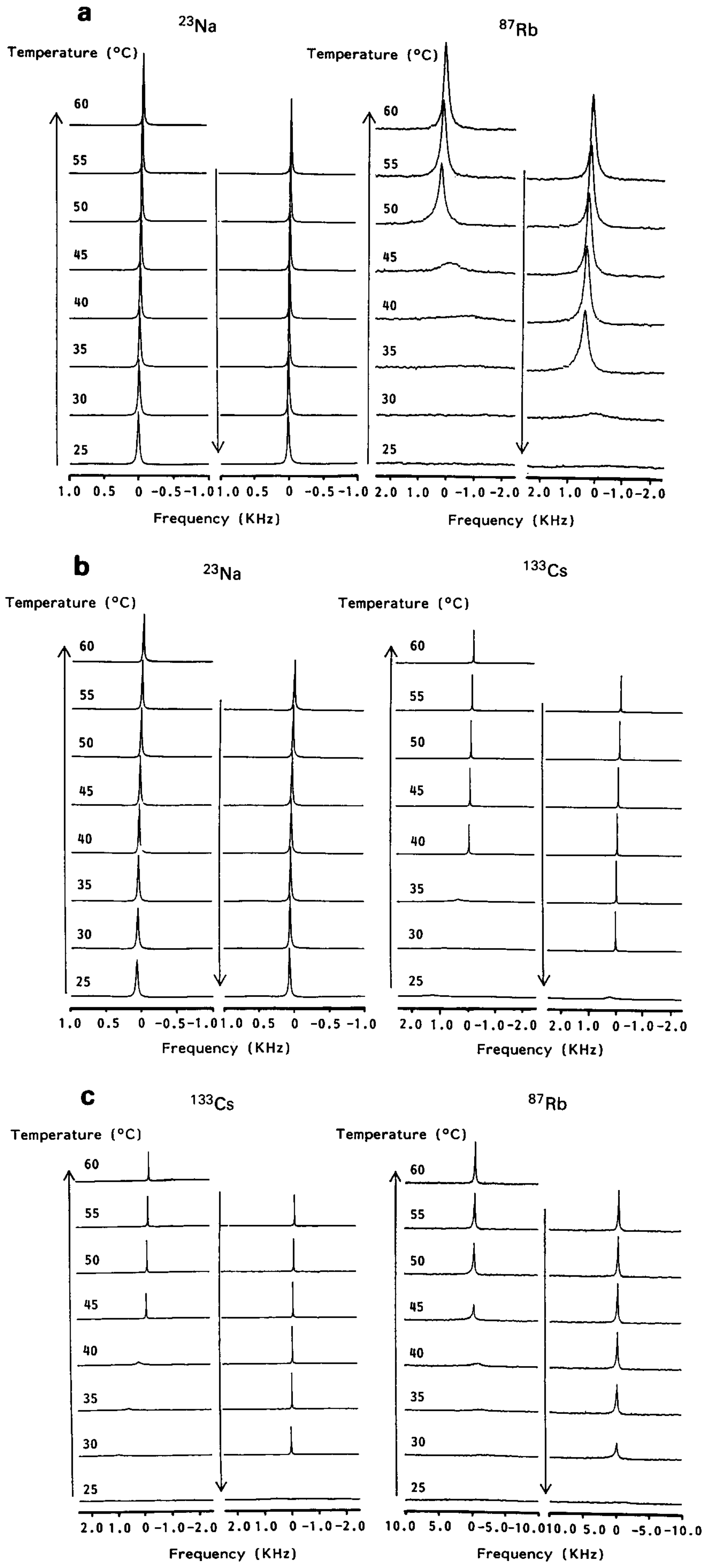

Figure 1 The temperature dependence of spectrum of ^{23}Na, ^{87}Rb and ^{133}Cs. (a) Sample I; (b) sample II; (c) sample III

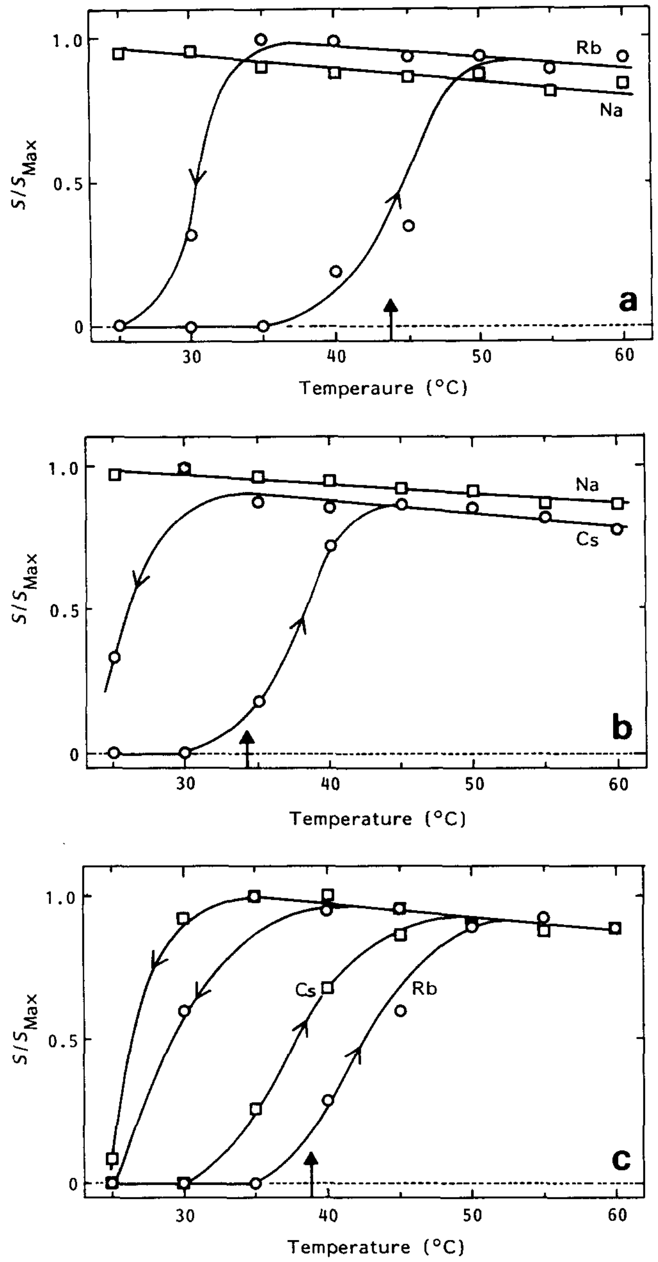

Figure 2 The temperature dependence of spectrum intensity of ^{23}Na, ^{87}Rb and ^{133}Cs. (a) Sample I; (b) sample II; (c) sample III

Figure 1. It is clear from this figure that the line shape of resonance spectra of ^{87}Rb and ^{133}Cs sharpen markedly with an increasing temperature while those of ^{23}Na are almost independent of temperature. The spectrum intensity, the spin–spin relaxation time and the chemical shift of these spectra are shown in *Figures 2*, *3* and *4* as a function of temperature. The arrows in these figures indicate the melting temperature of gel. It is found from these figures that during the heating process the n.m.r. parameters of ^{87}Rb and ^{133}Cs abruptly change in the vicinity of the melting temperature of gel. During the cooling process, these parameters under go a sudden change at lower temperature than occurs in the heating process, reflecting the onset of gelation. The exact gelation temperature during the cooling process could not be determined by the falling ball method because the tenuous gel structure in the early stage of gelation is easily destroyed by the ball.

The remarkable thermal hysteresis that was observed

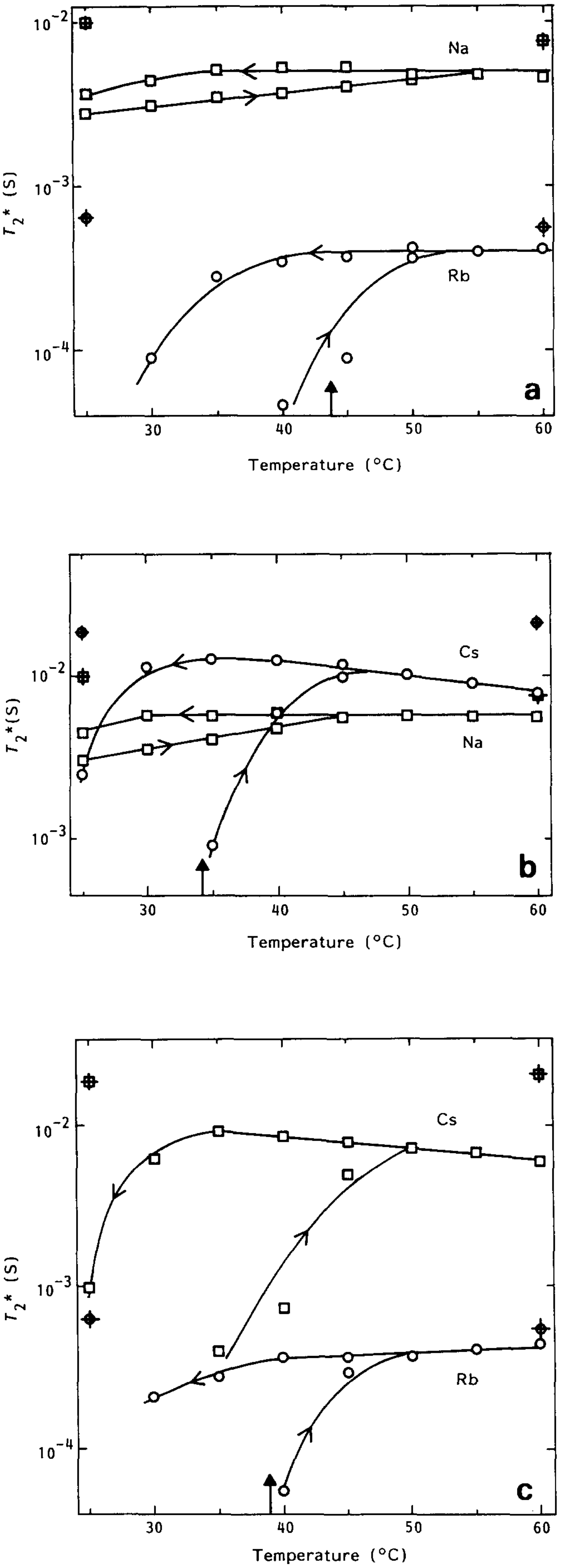

Figure 3 The temperature dependence of the spin–spin relaxation time of ^{23}Na, ^{87}Rb and ^{133}Cs. (a) Sample I; (b) sample II; (c) sample III. Symbols with cross mark indicate the spin–spin relaxation time of these group I cations in the salt solution at a concentration of 20 mM in the absence of κ-carrageenan

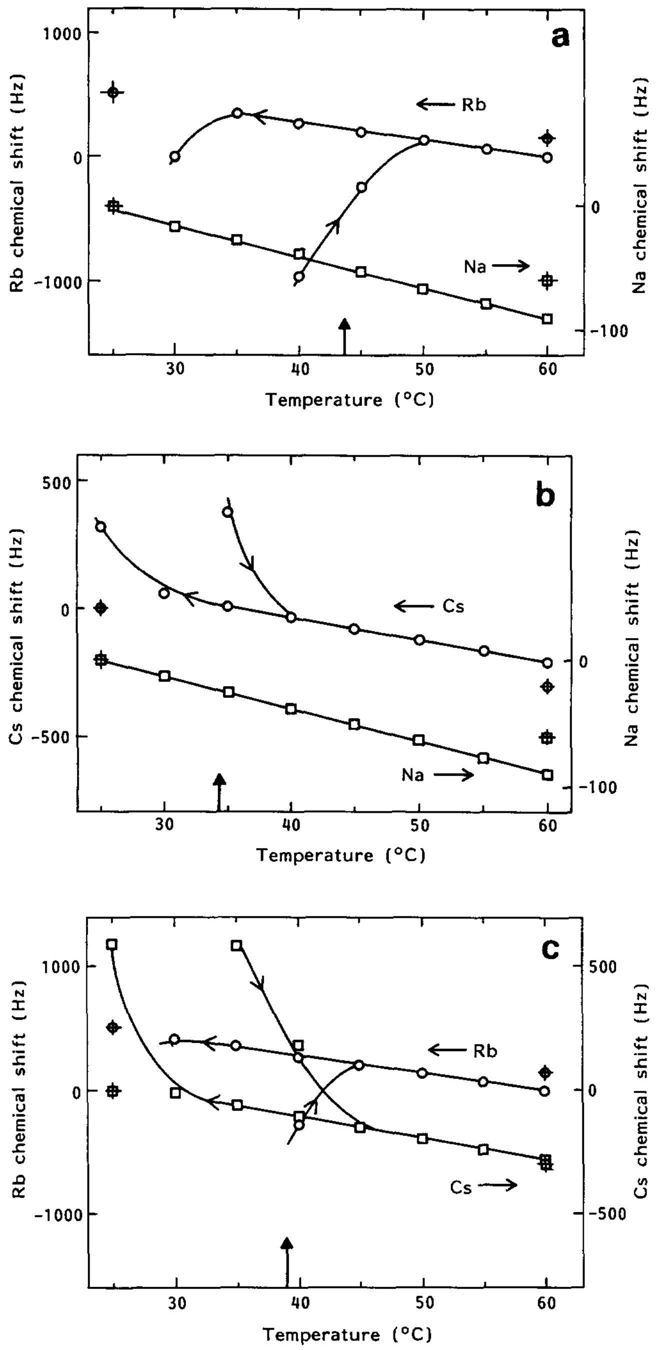

Figure 4 The temperature dependence of the chemical shift of ^{23}Na, ^{87}Rb and ^{133}Cs. (a) Sample I; (b) sample II; (c) sample III. Symbols with cross mark are the chemical shift of these group I cations in the salt solution at a concentration of 20 mM in the absence of κ-carrageenan

here in the temperature dependence of ^{87}Rb and ^{133}Cs resonance spectra has been observed by other experimental methods[1,2]. It is also of interest to note that ^{23}Na does not show any sudden change in n.m.r. parameters in the temperature range studied here. The small thermal hysteresis that was observed in the temperature dependence of the spin–spin relaxation time may be attributed to the thermal hysteresis of viscosity.

DISCUSSION

The motional state of observing nuclei affects the intensity and the line width of the spectrum[8]. A sharp resonance line is obtained when motion is fast. On the other hand, the high resolution spectrum cannot be observed when motion is severely restricted. A broad spectrum is obtained in the intermediate case. Thus, the spin–spin relaxation time obtained from the line width corresponds to that for mobile nuclei. The spectrum intensity indicates the number of mobile nuclei.

Abrupt changes in intensity and in the spin–spin relaxation time of gel-forming group I cations in the vicinity of the gelation temperature indicate that gel forming group I cations are hindered to move in the gel state, while these ions are mobile above the sol–gel transition temperature. On the other hand, Na ion does not show any singular behaviour in this temperature range. Furthermore, the spin–spin relaxation time of Na ion in samples I and II, in which Rb or Cs coexist, is almost the same as the one obtained from the aqueous solution of sodium chloride. This fact indicates that the non-gelling cation is free to move over the whole range of the temperature studied here.

These results strongly suggest that gel forming group I cations are bound to κ-carrageenan in the gel state, but that the Na ion does not bound to κ-carrageenan. It has been assumed that the binding of gel-forming cations to κ-carrageenan promotes the formation of junction zones. It has been assumed that the gelation of κ-carrageenan is accompanied by the conformational transition of polysaccharide chain from random coil to double helix, followed by the formation of cation (K^+, Rb^+ and Cs^+) mediated side-by-side aggregates (formation of junction zone) of double helices[1,9,10]. Several workers, however, assert a single helix model of gelation for κ-carrageenan system. In this model, it is also assumed that group I cations are bound to the junction zone that is formed by cation mediated side-by-side aggregate of single helices[11,12]. In either model, the line shape of n.m.r. spectra of gel-forming cations becomes broad when gel is formed because of the restriction of the molecular motion of gel-forming cations caused by the formation of junction zones. The results obtained here are in direct evidence of the selective interaction between the gel-forming group I cations and κ-carrageenan.

The chemical shift reflects the difference in magnetic environment of observing nuclei. The results shown in *Figure 4* indicate that the chemical shoft of gel-forming group I cations suddenly changes in the vicinity of the sol–gel transition temperature. On the other hand, the chemical shift of Na ion does not show any sudden change though it is slightly different from that of aqueous solution of sodium chloride. These results indicate that the environment of gel-forming group I cations which are surrounded by water and κ-carrageenan molecules is different between the gel state and the sol state, and the environment of non-gel-forming cations does not differ between the two states. These results also suggest the selective interaction between κ-carrageenan and gel-forming group I cations.

Further discussion of these results is difficult at present. It is, however, worth noting that the direction of change in chemical shift of Rb ion is opposite to that of Cs ion, i.e. Rb is more shielded and Cs is more deshielded when gel is formed. Detailed study of this phenomenon will probably give useful information about the structure of cross-linking points in κ-carrageenan gel that are believed to consist of group I cations and the ordered domain of κ-carrageenan.

It has been reported that the interaction with κ-carrageenan is different among gel-forming group I cations. Rb ion forms the most stable gel[1,2]. If we express the interaction energy of group I cations and κ-carrageenan molecules as E, the stability of cross-linking points is given by some function of E/kT where k is the Boltzmann constant. The melting temperature of the gel, T_m, is determined by the temperature at which $E/kT_m \simeq 1$. Hence, the melting temperature of the gel and the stability of the cross-linking points are directly related to the selectivity of group I cations[13].

The results obtained from sample III clearly indicate the difference in gel forming ability between Rb and Cs ions. In sample III, n.m.r. parameters of Rb change at higher temperatures than do those of Cs. This fact indicates that Rb ions bond more strongly to κ-carrageenan than do Cs ions. This suggests that they form more stable cross-linking points than do Cs ions.

The melting temperature of gel determined by the falling ball method indicates the disappearance of the infinite network. The infinite network disappears when the most stable cross-linking point in the gel melts. Hence, the melting temperature of gel agrees with the temperature at which n.m.r. parameters of Rb undergo a rapid change. The experimental results obtained here are, therefore, reasonably interpreted in terms of the difference in the selective interaction between κ-carrageenan and group I cations.

CONCLUSION

The temperature change of n.m.r. parameters of group I cations in κ-carrageenan solution clearly indicates the selective interaction between group I cations and κ-carrageenan molecule. The difference in the selective interaction among gel-forming cations can be demonstrated by the multinuclear magnetic resonance technique.

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