anisms to prevent wastage of gametes when the two species were artificially brought together. The two species do produce hybrids, but these hybrids obviously have reduced reproductive fitness, illustrated by difficulties in meiosis (*6*). We have here another instance of hybridization resulting from the interference of man. This appears to be the most common cause of hybridization in animals (*7*).

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8. Supported by NSF grants GB-2444 and GB-6944 to Dr. E. E. Williams, and Children's Bureau Project No. 906. We thank Mrs. Charlotte Kayavas and Miss Ida Leone for technical assistance, and Mr. Julius Boos for field aid. The manuscript was critically read by Dr. J. Wahrman.

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Proton Magnetic Resonance of Transfer RNA

Abstract. *The temperature dependence of the areas under the proton magnetic resonance spectra of unfractionated yeast transfer RNA in 1.0 molar NaCl is a consequence of salt-induced aggregation and does not constitute a monitor of the melting of secondary molecular structure. Such melting can be observed by following the widths of the resonances in the various regions of the spectra. Peaks attributable to dihydrouracil and the methyl groups of the methylated bases are detected in the spectra of unfractionated transfer RNA and alanine transfer RNA.*

Studies of the unfractionated transfer RNA (tRNA) from yeast by proton magnetic resonance (PMR) at 60 Mhz have indicated that melting of the secondary molecular structure can be followed by measuring the areas under the two broad, but separate, PMR peaks due to the protons in the bases and those at the 1′-positions in the ribose moieties (*1*, *2*). The interpretation was based on similar experiments with other polynucleotides which showed that only the PMR peaks from disordered regions were narrow enough to be observed (*3*). We have studied the 100 Mhz PMR spectra of unfractionated yeast tRNA at various salt concentrations as a function of temperature and conclude that the previously observed area changes were due to disassociation of salt-induced aggregates rather than to melting of molecular secondary structure.

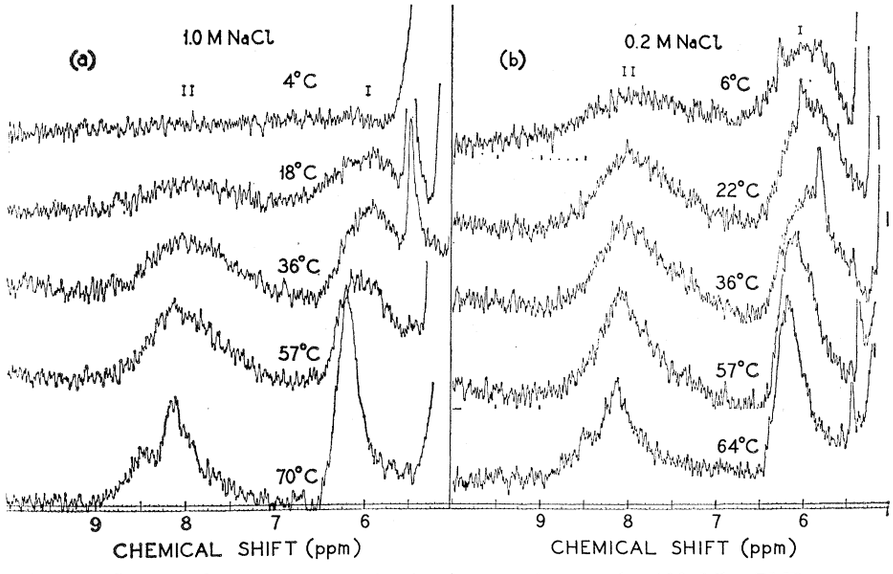

Fig. 1. Influence of temperature and salt concentration on the 100 Mhz PMR spectra of unfractionated tRNA from yeast, in D_2O, *p*D 6.8. The chemical shifts are measured in parts per million (ppm) from the external reference hexamethyldisiloxane present in a concentric capillary tube. The narrow peaks superimposed on region I are spinning sidebands from the intense HDO peak at 5 ppm.

In Fig. 1 we present the PMR spectra due to tRNA at 1.0*M* and 0.2*M* NaCl in D_2O, *p*D 6.8. These spectra were obtained on a Varian HA 100 spectrometer, operating in the internally locked, frequency-swept mode. The low-field peak (labeled peak II) centered on 8 ppm (parts per million from the external reference hexamethyldisiloxane) is due to the protons in the 2- and 8-positions of adenine, the 8-position of guanine, the 6-position of uracil and cytidine, and to most of the ring protons found in the unusual bases characteristic of tRNA. The higher field peak, labeled I, centered at 6 ppm, is due to all protons in the 1′-position of the ribose moieties and to the protons in the 5-position of uracil and cytidine. The drastic decrease in area with decreasing temperature for the 1.0*M* NaCl solution (Fig. 1a) is similar to that found by McDonald *et al.* (*1*), with the exception that the areas of peaks I and II behave identically (Fig. 2). Consideration of the average base composition of unfractionated tRNA from yeast (*4*) indicates that the area under peak I should be approximately 25 percent greater than that under peak II if all protons are mobile enough to be observed. This condition was satisfied for all the samples studied (within experimental error; Fig. 2). Therefore, we have no evidence for the involvement of the ribose protons in bonds stronger than those experienced by the base protons. The sugar protons appear to be liberated at the same temperature as the bases, as one would expect if the principal source of double-strand stabilization were base-base interaction.

However, the PMR behavior shown in Fig. 1b is obtained at all NaCl concentrations below 0.75*M*. No dramatic changes in area occur from 5° to 85°C, and the resonances are easily observable even at 3°C. The 0.5*M* NaCl solution behaves like the 1.0*M* solution, if it is brought up to 0.1*M* in $MgCl_2$.

The PMR spectra of both samples in Fig. 1 show an improved resolution above 55°C. Presumably at these temperatures disaggregation is complete even in the 1.0*M* NaCl samples, and

one observes better-resolved PMR spectra because of the melting of the secondary structure of the individual tRNA molecules. At all the salt concentrations studied (0.0065*M* to 1.0*M*, *p*D 7 ± 0.2, with or without Mg^{++} at concentrations from 0.01*M* to 0.1*M*), the resolution improvement always occurred over the temperature range 55° to 70°C, with the most significant improvement occurring at approximately 63°C.

Schleich and Goldstein (*5*) have found that the tRNA of *Escherichia coli* is highly aggregated in 1.0*M* NaCl. To demonstrate the presence of aggregates in our preparations, we have run elution profiles on Sephadex G-100 under the same conditions (100 mg of tRNA per milliliter, 1.0*M* or 0.1*M* NaCl, *p*H 6.6) as the PMR experiments. With 1.0*M* NaCl only a strong front-running peak was observed, whereas with 0.1*M* NaCl a weak front-running peak and a strong later-running peak were manifest. The later-running peak coincided with that usually found in 0.1*M* salt for much lower tRNA concentrations. Thus, it appears that, even in 0.1*M* NaCl, there are some aggregates present at the high concentrations of tRNA used for PMR experiments, while at 1.0*M* salt nearly all of the tRNA is aggregated. This accounts for the slight increases in area observed over the temperature range 5° to 20°C for samples with salt concentrations less than 0.75*M* (Fig. 2). Further evidence for aggregation is given by the relative sedimentation rates of unfractionated tRNA (100 mg/ml, *p*H 6.6) in 1.0*M* and 0.1*M* NaCl; in 1.0*M* NaCl the rate is considerably faster than in 0.1*M* NaCl.

We have prepared a sufficient quantity of purified alanine tRNA from yeast to study its PMR behavior. In 0.1*M* NaCl, *p*D 7.0, the areas of regions I and II exhibited temperature dependences similar to those shown in Fig. 2 for 0.02*M* NaCl and 0.0065*M* $MgCl_2$. This demonstrates that the behavior observed for unfractionated yeast tRNA is not due to the presence of impurities introduced by the method of preparation.

In the PMR spectra of unfractionated tRNA at 30°C, it is possible by use of computer averaging to detect very weak broad peaks at 3.07 and 3.90 ppm. Raising the temperature to 67°C results in the appearance of narrow peaks (2 to 5 hz) at 3.94, 3.86, 3.73, 3.25, 3.15, 3.09, 3.03, 2.57, and 2.12 ppm. These correspond to the positions found for the methyl and dihydro groups of the uncommon bases found in tRNA (*6*). With purified alanine tRNA, the high temperature peaks are at 3.80 and 3.10 ppm. The odd bases occur in the loop regions of the proposed cloverleaf model for tRNA (*7*), and thus these high-field resonances provide a valuable structural probe.

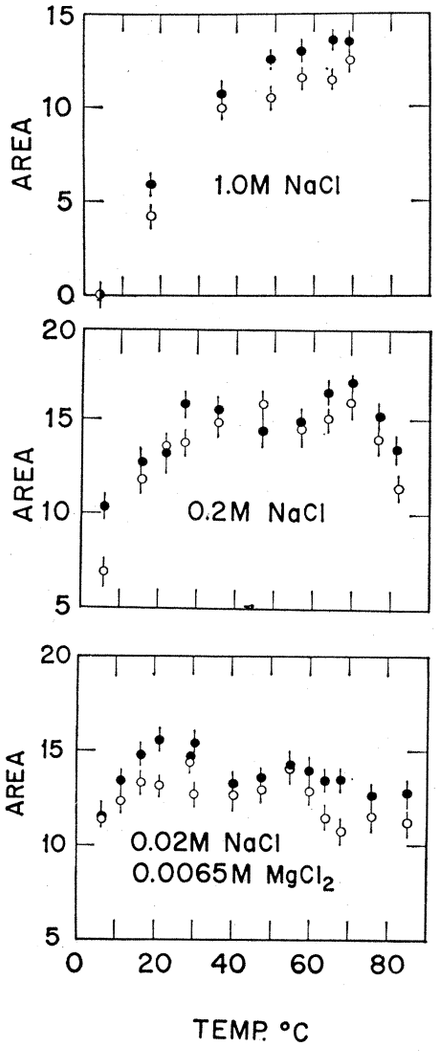

Fig. 2. Variation of the areas under peaks I and II of the 100 Mhz PMR spectra of tRNA with temperature. The filled circles represent peak I (protons in the 1′-position of the ribose moieties and the 5-position of uridine and cytosine); the open circles represent peak II (base protons). The ordinates of the upper plate differ from those in the lower two plates.

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Calcergy Inhibited by Calciphylactic Challengers

Abstract. *The subcutaneous calcification effected in the rat at sites directly treated with calcergens, such as lead acetate, $CeCl_3$, $CaCl_2$, and $KMnO_4$, is inhibited by simultaneous local application of various calciphylactic challengers, but not by many other compounds.*

Calciphylactic challengers produce calcification of soft tissues at the sites of their administration only after sensitization by systemic treatment with such calcium-mobilizing factors as parathyroid extract, vitamin D, or dihydrotachysterol; calcergens exert the same effect without any previous sensitization. Thus calciphylaxis is, while calcergy is not, dependent upon sensitizing alterations in systemic calcium and phosphate metabolism. However, the end results of both reactions are the same: deposition of hydroxylapatite which forms a readily visible white disk at the site of injection of the provocative substance (*1*).

Recently we observed that topical calcification is prevented in rats if calcergens are injected simultaneously with calciphylactic challengers, whereas agents that do not cause calcification upon subcutaneous injection, either in unpretreated or in calciphylactically sensitized rats, fail to protect against the topical calcium deposition normally elicited by calcergens. This apparently paradoxical fact further emphasizes the essential dissimilarity of calciphylaxis and calcergy.

Female Sprague-Dawley rats averaging 100 g (90 to 110 g) were divided into groups each of 5 to 10 animals and injected subcutaneously under the