polymerase activity by actinomycin D and DNAase has no effect on the control rate of amino acid incorporation into protein or the thyroxine effect. Thyroxine also stimulates the rate of poly U-directed incorporation of phenylalanine into polyphenylalanine. These results are interpreted to indicate that thyroxine stimulates amino acid incorporation into microsomal protein independently of any action on DNA-dependent RNA polymerase activity or messenger RNA synthesis.

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*THE EFFECT OF TEMPERATURE ON THE PROTON MAGNETIC RESONANCE SPECTRA OF RIBONUCLEASE, OXIDIZED RIBONUCLEASE, AND LYSOZYME**

BY MORTON MANDEL

DEPARTMENT OF GENETICS, STANFORD UNIVERSITY SCHOOL OF MEDICINE

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The proton magnetic resonance spectra of ribonuclease,[1, 2] oxidized ribonuclease, and lysozyme[3] have been studied as a function of temperature. In ribonuclease, the structure of that part of the spectrum arising from the aromatic ring protons changes in the temperature range 50–70°C. At 70°C and above there is significant narrowing of some of the spectral lines; in contrast, the proton spectrum of lysozyme is apparently unaffected by temperature changes to 75°C. The spectral lines of oxidized ribonuclease are much narrower than those of ribonuclease at room temperature. Higher temperature produces some additional narrowing but otherwise the spectrum remains unchanged up to 90°C.

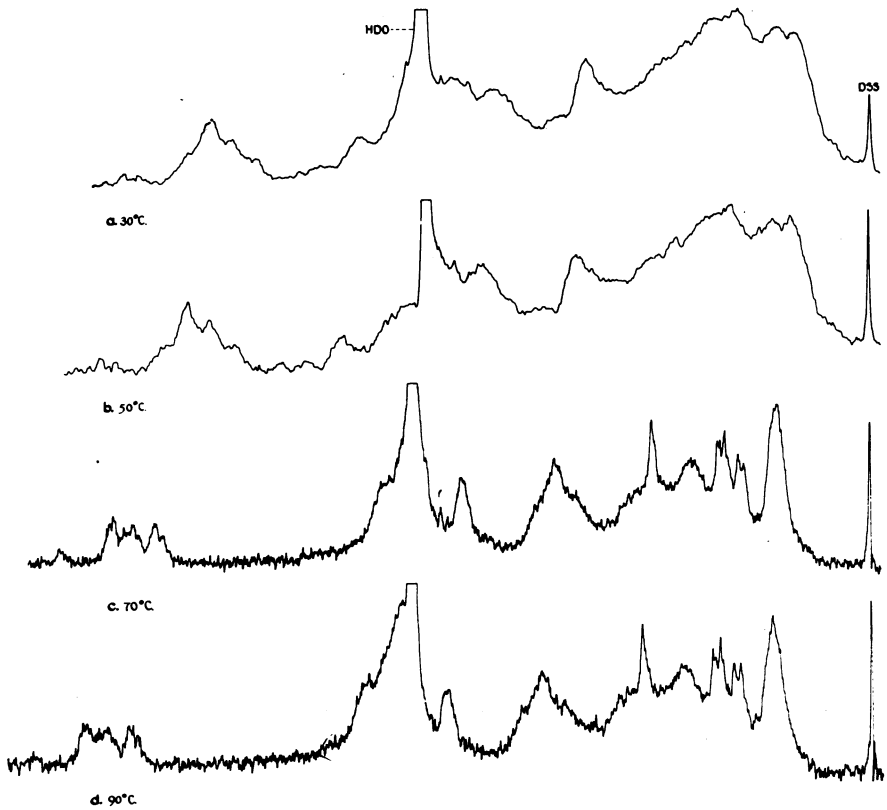

FIG. 1.—Spectra of a 5% solution of ribonuclease in D_2O at pH 4.5 as a function of temperature. Chemical shift of HDO resonance is temperature-dependent, moving up field with increasing temperature.

The spectra of ribonuclease and oxidized ribonuclease were observed in a Varian H100 N.M.R. spectrometer operating at a fixed frequency of 100 Mc with 2-kc modulation and phase sensitive detection. A special temperature probe[4] controlled the temperature to ± one degree.

The lysozyme spectrum was observed in a Varian H60 apparatus with a standard temperature probe. The 30°C temperature spectra are calibrated, and chemical shifts[5] can be measured directly; however, the significance of the spectra lies not in the absolute values of the chemical shift but in relative changes of position and line widths. Sodium 2,2-dimethyl-2 sila pentane-5 sulfonate (DSS) was used as an internal reference standard for all chemical shift measurements.

Ribonuclease (type XII-A ribonuclease "A" powder) from Sigma Chemical Company, lysozyme (eggwhite, 3 × crystallized) from Sigma Chemical, and oxidized ribonuclease from Mann Research Laboratories were dissolved in D_2O supplied by Bio-Rad Labs and then evaporated to dryness. After this procedure was repeated several times, a 5 per cent solution of each material was made in 0.5 ml of D_2O.

Discussion and Results.—Ribonuclease spectra at 30, 50, 70, and 90°C are shown in Figure 1. The pH of the ribonuclease solution was 4.5 and varied very slightly

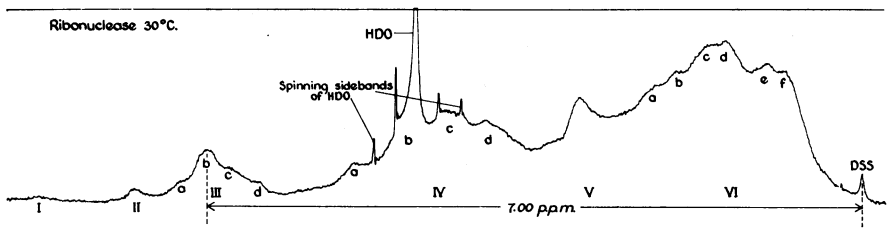

FIG. 2.—Spectrum of ribonuclease under same conditions as Fig. 1, obtained with a more sensitive room temperature probe.

over the above temperature range. A ribonuclease spectrum obtained with a more sensitive normal probe at 100 Mc and 30°C is shown in Figure 2. The peaks are designated in the manner described previously by the author, who has correlated the spectra of the individual amino acids with the spectral lines of ribonuclease.[2] Peak III at a chemical shift of −7.00 ppm arises mainly from the aromatic residues. In the temperature range 50–70°C there is a marked change in the intensity pattern of this peak (see Figure 1). The high field region of the multiplet (IIIc) grows in intensity relative to the low field side (IIIa).

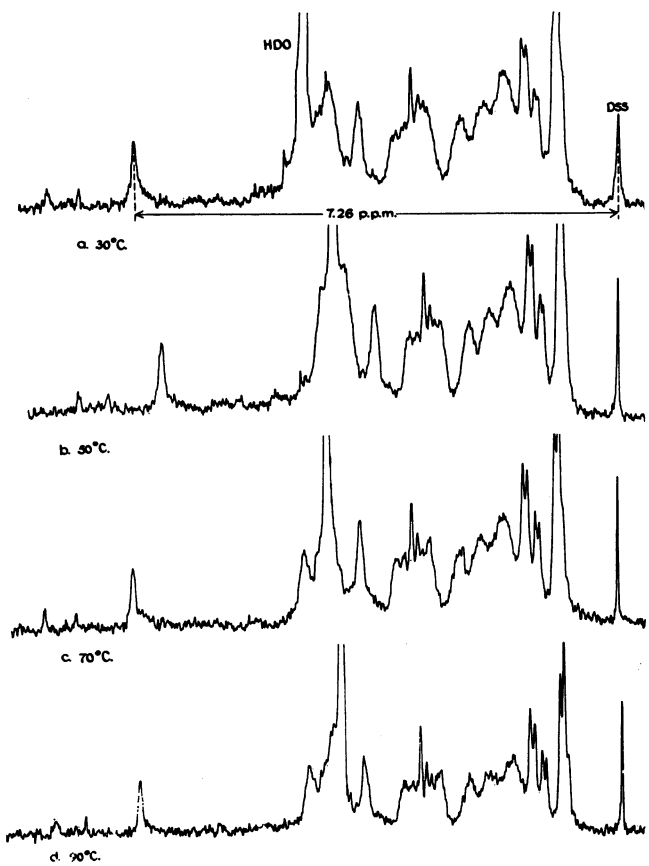

FIG. 3.—Spectra of a 5% solution of oxidized ribonuclease in D_2O at pH 3.4 as a function of temperature. Chemical shift of HDO resonance is temperature-dependent.

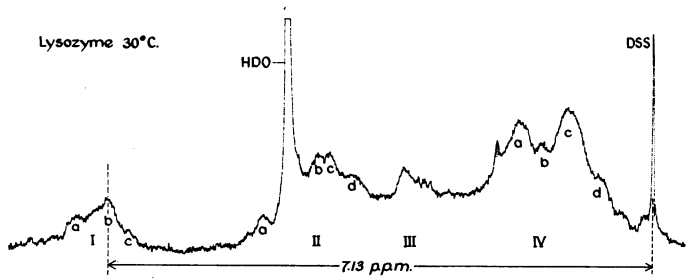

FIG. 4.—Room temperature spectrum of 5% solution of lysozyme in D_2O at pH 5.4 obtained at a frequency of 100 Mc.

At 70°C there is some line narrowing, mainly in that part of the spectrum arising from the methyl groups of aliphatic amino acids (peak VI). The unfolding of the protein would be expected to allow increased motion of the side chains. It is well known in n.m.r. theory that motion (rotation and translation) reduces the width of a resonance line.[6]

According to Hermans and Scheraga[7] the transition temperature for ribonuclease

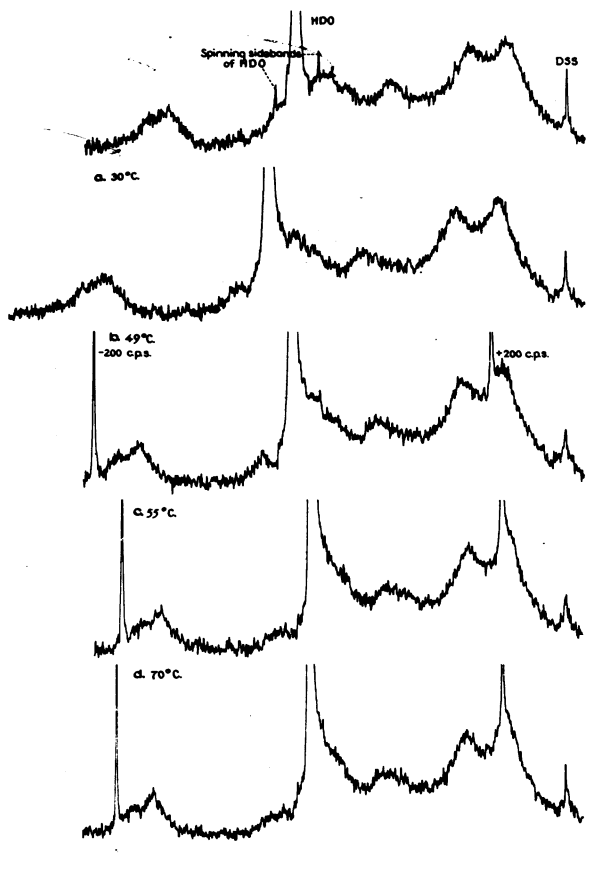

FIG. 5.—Spectra of a 5% solution of lysozyme in D_2O at pH 5.4 as a function of temperature at 60 Mc. The peaks labeled ±200 cps are side bands of the HDO line used for calibration purposes.

at pH 4.5 is 58°C, which is consistent with the n.m.r. data. A comparison of the high temperature spectrum of ribonuclease (Fig. 1*c*, *d*) with the 30°C spectrum of oxidized ribonuclease at pH 3.4 (Fig. 3) shows that the extent of unfolding responsible for this transition is not as great as that occurring upon oxidation.

In oxidized ribonuclease there is much more motional narrowing of the methyl protons. In addition there is pronounced motional narrowing of the aromatic protons (−7.26 ppm in Fig. 3*a*) and some narrowing of protons situated near the peptide backbone (in the range of −3.00 ppm to −5.00 ppm). The small decrease in line width of oxidized ribonuclease at high temperatures is due to small changes in viscosity and to increased thermal motion.

The change in pattern of the aromatic peak in the thermal transition of ribonuclease is probably associated with the six tyrosine residues. In aqueous solutions, three of these ionize normally and reversibly between pH 9 and 11.5, and three ionize above pH 12, a condition which alters the conformation of the molecule irreversibly.[8, 9] However, in heat denaturation only two of the three abnormal tyrosines are normalized according to ultraviolet spectral data.[10] These results suggest that some of the tyrosyl groups may interact with other side chains in the native molecule through hydrogen bonding. In contrast the n.m.r. spectrum of the six tyrosines seem to be normal both in ribonuclease at room temperature and in oxidized ribonuclease. It is quite possible that hydrogen bonding has no effect on the chemical shift of the tyrosine ring; however, as hydrogen bonds break with increasing temperature, restrictions on the motion of the aromatic rings are removed. Increased freedom of movement can permit a different environment resulting in a different chemical shift for the aromatic protons. In ribonuclease, the three abnormal tyrosines at positions 25, 73, and 97 in the sequence[10] are adjacent to disulfide bridges, whereas in oxidized ribonuclease where the disulfide bridges are broken, all six tyrosines titrate normally and also have similar n.m.r. spectra.

The spectrum of lysozyme at room temperature and pH 5.4 (Fig. 4) is indicative of a compact globular molecule of roughly the same size as ribonuclease based on line width measurements. Examination of the spectra at higher temperatures (Fig. 5) reveals no apparent change in the structure of the aromatic peak (7.13 ppm in Fig. 4). Even at 75°C there is no line narrowing, indicating that in lysozyme, the disulfide bridges together with noncovalent forces probably hold the molecule in such a rigid conformation that increased motion of side chains is not possible.

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INTERFERON PRODUCTION INDUCED BY STATOLON

BY W. J. KLEINSCHMIDT, J. C. CLINE, AND E. B. MURPHY

LILLY RESEARCH LABORATORIES, INDIANAPOLIS, INDIANA

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Early in the investigations on interferon, Isaacs and Lindenmann obtained suggestive evidence that the nucleic acid of the virus was responsible for the stimulation of interferon production in cells.[1] Recently, Rotem, Cox, and Isaacs[2] reported that RNA[3] of animal source, heterologous to the tissue in which it was tested, was also able to produce interferon. This observation provided an explanation for the results of O'Dell, Wright, and Bieter,[4] who as early as 1953 showed that yeast RNA inhibited MM virus in mice.

In our laboratory we have continued the investigation of the properties of statolon, an antiviral agent produced by the mold *Penicillium stoloniferum*.[5] It has proved to be prophylactically active against a wide spectrum of viruses in tissue culture and in animals.[6-13] Statolon is a complex anionic polysaccharide with a relatively high content of galacturonic acid, which is the primary contributor to its polyanionic character.[5]

Structurally, RNA also is a polyanion. The finding by Rotem *et al.*[2] that heterologous RNA stimulated the production of interferon suggested that the mechanism of action of statolon possibly lay in its capacity to stimulate the production of interferon. This possibility has been investigated, and the results presented in this paper show that statolon induces the production of a viral inhibitor in cells, and that this inhibitor has the properties of interferon.

Materials and Methods.—Statolon: A freeze-dried preparation was used that contained 89.47% sodium bicarbonate and glucose, in equal proportion.[14] The statolon was dissolved in water, 50 mg/ml, and the pH adjusted to 7.5 with CO_2. The solution of statolon was diluted 1:300 with medium 199, bringing the concentration of active statolon to 17.5 μg/ml.

Virus: Vaccinia virus, strain V-1, was obtained through the kindness of Dr. J. Lindenmann, University of Florida. Stock virus was prepared in HeLa cells and was stored in sealed glass ampoules at −60°C.

Cells: Primary cultures of chick embryo (CE) cells from 11-day-old embryos were employed.

Production of induced inhibitor: 70×10^6 CE cells in 30 ml of medium 199 containing 4% calf serum were added to rectangular pint bottles with a surface area of 100 cm^2 and incubated 48 hr. The medium was then replaced with 30 ml of a statolon solution (17.5 μg/ml), the cells were incubated another 24 hr, and the fluid was harvested. Residual statolon was removed from the fluid by concentrating it fivefold in dialysis bags by means of polyethylene glycol, then lowering the pH to 4.5 and centrifuging for 90 min at 105,000 $\times$ g in the Spinco (Model L) centrifuge. The supernatant fluid was removed from the tubes and the pH was adjusted to 7.3. Assays showed that statolon solutions that had never been in contact with cells no longer possessed inhibitory activity after such treatment. After removal of the statolon, fluids were diluted 1:5 to the initial