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Some Aspects of the Effect of Deuterium Oxide (D₂O) Substitution for Water (H₂O) on the Helical Stability of Yeast Transfer Ribonucleic Acid

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Substitution of D₂O for H₂O in aqueous media has been shown to be capable of enhancing helical stability in DNA, polyribonucleotides and *Escherichia coli* ribosomes over certain pH ranges and at low ionic strength (Lewin, 1966, 1967a,b; Lewin & Williams, 1971). We have therefore examined spectrophotometrically the effect of the substitution on yeast tRNA prepared by the Holley (1963) method freed from protein contamination.

In solutions containing 0.033 g of tRNA/l and of ionic strength 10^{-3} (prepared and kept under nitrogen free from oxygen and preincubated for 17 h at 3°C) D₂O substitution resulted in an increase of several degrees Celsius in the T_m value ('melting' temperature) in the approximate pH/pD region 5.9, as well as in the alkaline pH region, but without any significant difference in the neutral pH region. Extinction comparisons at both pH/pH and pH/pD scale variations showed increased hypochromicity on D₂O substitution over most pH regions. The T_m differential decreased with increasing ionic strength and disappeared at 10^{-2} ionic strength and above. λ_{max} and λ_{min} varied somewhat with pH and ionic strength; the substitution caused a shift to a lower wavelength by approx. 0.5 nm in λ_{max} ; a parallel shift of approx. 0.5 nm was found in the trough region at about 230 nm. Our low-ionic-strength-D₂O pro-helix results can be collated with those of Mahler, Dutton & Mehrotra (1963), which showed only slight D₂O pro-helix effects with tRNA; they used standard $0.01 \times$ saline-citrate plus 1 mM-Mg^{2+} , which is a neutral pH medium of ionic strength approx. 10^{-2} , conditions that we

found to lower the D₂O pro-helix effect to almost non-significant proportions.

Our results and other relevant observations in the substitution of D₂O on yeast tRNA can be understood within the overall framework (suggested by Lewin, 1971b) that includes the following: (1) increased O-D...O bond strength as compared with that of O-H...O (Nemethy & Scheraga, 1964; Lewin, 1967a,b); (2) decreased differential of the O-D...O bond strength in relation to that of O-H...O with rise in ionic strength (Lewin, 1967a,b); (3) higher structural order of D₂O than that of H₂O (e.g. Nemethy & Scheraga, 1964), which results in enhanced extrusion of water involved in helix formation (Lewin, 1970, 1971a,b).

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Radioactive Labelling of Multiple-Drug-Resistance Factor in *Escherichia coli*

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Freifelder & Freifelder (1968) have described a technique for labelling specifically the DNA of the F-factor and F-merogenotes in *Escherichia coli* after transfer of the plasmids during conjugation. The method has been adapted by Silver & Falkow (1970) and in the present experiments for labelling the DNA of a fertility-de-repressed multiple-drug-resistance factor (R-factor) designated Rldrd19 (Meynell & Datta, 1966).

A strain of *E. coli* unable to utilize exogenous thymine was used as a donor of the de-repressed R-factor and mated with a thymineless recipient strain that is unable to excise u.v.-induced pyrimidine dimers in DNA and that had been previously killed by u.v. irradiation. During the first 30 min of mating [³H]thymine was incorporated into the trichloroacetic acid-insoluble fraction, in excess of that found when the donor strain, into which the

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