Effects of dilute HCl on yeast tRNA^{Phe} and E. coli tRNA^{fMet}₁

Jane E. Ladner[‡] and Martin P. Schweizer^{*}

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, 2727 Campus Drive, Irvine, California 92664

Received 19 November 1973

ABSTRACT

HCl treatment of yeast tRNA^{Phe} under conditions generally used for excision of 'Y' base results in structure and conformation changes as monitored by line widths in the PMR spectra at 220 MHz and by optical rotation. Like exposure of E. coli tRNA^{fMet}₁ causes similar changes in the PMR spectra and optical rotation although no residues are eliminated. Electrophoresis in polyacrylamide gels provides evidence for aggregation in HCl-treated tRNA^{fMet}₁. One must thus consider a general effect of HCl exposure as well as possible residue removal in assessing induced structural and conformation changes in tRNA.

INTRODUCTION

Exposure of yeast tRNA^{Phe} to dilute HCl, (Thiebe and Zachau 1968, 1970), results in excision of a fluorescent, highly substituted guanine base, the structure having recently been elucidated (Nakanishi *et al*, 1970). The product, tRNA^{Phe}_{HCl}, has been found, by a variety of spectral techniques, to have altered conformational properties (Thiebe and Zachau, 1970). Evidence for perturbed conformation of the anticodon loop has been obtained from studies of complimentary oligonucleotide binding to tRNA^{Phe}_{HCl} (Pongs and Reinwald, 1973). Additionally, tRNA^{Phe}_{HCl} displays altered codon response (Thiebe and Zachau, 1970; Ghosh and Ghosh, 1972), as well as somewhat different amino acid charging behavior (Thiebe and Zachau, 1970).

Abbreviations: NMR, nuclear magnetic resonance;
pmr, proton magnetic resonance.

The influence of modified nucleosides on solution properties of tRNA, using chiefly ^1H -nmr at 220 MHz, has been actively pursued in our laboratories (Crawford et al, 1971; Schweizer et al, 1973). Resonances due to methyl and methylene groups of the modified residues are readily observed and assigned (Crawford et al, 1971) particularly if the tRNA is examined in organic solvents such as DMSO, which lessen dipolar interactions between the monomer units. In an attempt to assign the proton resonances due to the "Y"-base, we have examined the 220 MHz spectra of yeast tRNA^{phe} before and after exposure to dilute HCl. In theory, one should be able to identify the Y-specific protons as those missing in the spectra of tRNA^{phe}_{HCl}. E. Coli tRNA^{fMet}₁ has also been studied as a control, having no groups labilized by this treatment.

EXPERIMENTAL

Materials and Methods

Baker's yeast tRNA^{phe} (phenylalanine acceptance, approximately 1000 pmoles/A₂₆₀ unit) was prepared using BD cellulose columns (Wimmer et al, 1968). tRNA^{fMet}₁ (methionine acceptance, 1615 pmoles/A₂₆₀, formyl acceptance 1690 pmoles/A₂₆₀) from E. Coli K12MO (Lot #15-290) was obtained from Dr. A. D. Kelmers, Oak Ridge National Laboratory. We thank the National Institute of General Medical Sciences for this material. DMSO-d₆ and D₂O, both 100 atom %d, were obtained from Diaprep, Inc.

To insure solubility in DMSO, lithium salts of the tRNA samples were prepared by passage through Dowex 50 (Li). HCl treatment of both yeast tRNA^{phe} and E. Coli

tRNA₁^{fMet} was performed according to Thiebe and Zachau (1968). 28 mg yeast tRNA^{phe} and 10 mg E. Coli tRNA₁^{fMet} were respectively dissolved in 1 ml distilled water. 1 N HCl was added to pH 2.9. After incubation at 37°C for 4-5 hr. the solutions were carefully neutralized with 0.1 N LiOH at room temperature, dialyzed to remove LiCl and lyophilized. NMR samples were prepared by placing the appropriate weight of lyophilized tRNA (lithium salts) in a 5 mm tube equipped with a stopcock, which was attached to a vacuum manifold. D₂O was distilled into the tube and the tRNA dissolved at room temperature. DMSO was subsequently added and the tube sealed off. All tRNA samples were examined in the mixed solvent 67% v/v DMSO-d₆/D₂O. No concentration dependence was observed in this solvent.

Instrumentation

Pmr spectra were obtained on a Varian HR-220 spectrometer in conjunction with a 1024 signal averager. Optical rotations were measured on a Perkin-Elmer Model 141 using 1 decimeter cells of 1 ml volume. Observed rotations obtained for the tRNA samples as a function of volume % DMSO were all corrected for contributions from the corresponding mixed solvent. Samples for this study were prepared by adding the tRNA in water to the appropriate water-DMSO mixtures, constituting a total volume of 1 ml.

RESULTS AND DISCUSSION

In Figure 1 is displayed time averaged 220 MHz spectra of the aromatic proton region of the various tRNA samples in the mixed solvent. We have selected these solution conditions for the comparative pmr study because

it was previously shown (Crawford et al, 1971; Schweizer et al, 1973) that tRNA is denatured by DMSO to an extended random coil, thus minimizing dipolar interactions between the monomer units and permitting examination of the proton resonances from individual nucleotide classes. In D₂O alone aromatic proton line widths from untreated yeast tRNA^{phe} are about 50 Hz while various methyl protons are 20-25 Hz (Crawford et al, 1971), thus one is unable to assign resonances as well as assess possible linewidth changes as a result of the described treatments.

It is apparent from the line broadening in the spectra of the HCl treated tRNAs that significant alteration has occurred in both cases. The high field regions where methyl and methylene protons absorb were also broadened, so that no information regarding the assignment of Y-base resonances could be obtained. Nevertheless, it is instructive to note the similar effect of HCl upon both tRNA molecules. Bulk yeast tRNA exposed to acid in this fashion yielded the same results. Obviously, since no excision of a large hydrophobic moiety has occurred in the case of the tRNA₁^{fMet} the effect noted must solely arise from acid induced conformational changes for this tRNA. The possibility of line broadening due to the presence of paramagnetic ions in the HCl treated samples was eliminated by noting that added EDTA caused no line narrowing. Minute amounts of LiCl, resulting from neutralization of the HCl, and remaining after the exhaustive dialysis, was not the source of the observed effects, since LiCl added to the tRNA₁^{fMet} sample in the NMR tube caused no linewidth increases.

Figure 1 - Computer time averaged 220 MHz spectra [tRNA^{phe} and $\text{tRNA}^{\text{phe}}_{\text{HCl}}$, 30°C ; $\text{tRNA}^{\text{fMet}}$ and $\text{tRNA}^{\text{fMet}}_{\text{HCl}}$, 17°C] of aromatic proton regions (250 Hz) for various tRNA samples. Top left: 80 mg/ml yeast tRNA^{phe} (9 scans). Bottom left: 80 mg/ml $\text{tRNA}^{\text{phe}}_{\text{HCl}}$ (10 scans). Top right: 33 mg/ml E. Coli $\text{tRNA}^{\text{fMet}}$ (100 scans). Bottom right: 33 mg/ml E. Coli $\text{tRNA}^{\text{fMet}}_{\text{HCl}}$ (100 scans). Peak assignments, as previously determined (Crawford *et al*, 1971) for tRNA^{phe} are from left to right; H-8 of adenine, H-2 of adenine, H-8 of guanine, and H-6 of cytidine and uridine.

Figure 2 - Specific rotation, $[\alpha]_{365}^{25}$ of tRNA^{phe} and tRNA^{hcl} as a function of volume percent organic solvent. tRNA^{phe}: -●-, DMSO; -○-, ethylene glycol; tRNA^{hcl}: -▲-, DMSO; -□-, ethylene glycol. Data taken on a Perkin-Elmer Model 141 polarimeter in a one decimeter cell (1 ml).

The tRNA^{fMet}₁(HCl) was heated at 60° C for 10 min. in 20 mM MgSO₄ then slow cooled. No change was apparent upon re-examination in the mixed solvent.

Since both treated and untreated tRNAs were examined in the solvent-denatured state, it is apparent that the acid exposure has resulted in altered structures such that the denaturing effect of the DMSO is not as extensive, hence the greater linewidths in the pmr spectra.

The differences in effect of organic solvent upon tRNA conformation before and after HCl treatment may also be followed by optical rotation as is illustrated in Figure 2. Here the specific rotation, $[\alpha]_{365}^{25}$, for tRNA^{phe} and tRNA^{phe}_{HCl} as a function of added DMSO and ethylene glycol is presented. It may be seen that conformational changes caused by HCl are reflected in aqueous media, as well as in the response to the denaturing influence of the organic solvent. The large positive rotation for tRNA^{phe}, which is normally observed for ordered tRNAs in aqueous media (Reeves *et al*, 1970; Fasman *et al*, 1965), has been reduced by one third in tRNA^{phe}_{HCl}. In addition, since the rotation does not change to negative values as seen for the untreated material, this acid treated polymer is evidently not as completely "melted-out" upon DMSO addition as is tRNA^{phe}, a conclusion reached above in discussion of the pmr results.

Ethylene glycol is not as effective a denaturing agent as is DMSO. In an ORD study Fasman, *et al* (1965) showed that the extent of bulk yeast tRNA denaturation by 90% ethylene glycol was similar to 90° C in aqueous buffer. We have previously shown (Crawford *et al*, 1971) that DMSO

is considerably more effective than thermal means in denaturing tRNA.

Specific rotations, $[\alpha]_{365}^{25}$, for $\text{tRNA}_{1}^{\text{fMet}}$ and $\text{tRNA}_{1(\text{HCl})}^{\text{fMet}}$ in aqueous media exhibit similar behavior as $\text{tRNA}_{1}^{\text{phe}}$ and $\text{tRNA}_{\text{HCl}}^{\text{phe}}$, although the reduction in positive rotation for $\text{tRNA}_{1(\text{HCl})}^{\text{fMet}}$ is 13-15% compared with 30% in $\text{tRNA}_{\text{HCl}}^{\text{phe}}$. The less drastic change in rotation for $\text{tRNA}_{1(\text{HCl})}^{\text{fMet}}$ may be due to aggregate formation which is indicated by bands moving slower than the main tRNA band upon electrophoresis in 10% polyacrylamide gels. Support for this idea was obtained by re-running the gel electrophoresis in the presence of 4 M urea. Under these dissociative conditions the $\text{tRNA}_{1}^{\text{fMet}}$ and $\text{tRNA}_{1(\text{HCl})}^{\text{fMet}}$ mobilities were the same. No evidence for aggregate formation was found in the electrophoresis of $\text{tRNA}_{\text{HCl}}^{\text{phe}}$.

The pmr and rotation data presented here point out that mild HCl treatment as is commonly used to excise "Y" bases from various tRNA^{phe} species causes conformational and structural changes which are manifest whether or not a particular residue is cleaved. It is highly unlikely that the 7-methylguanosine in the $\text{tRNA}_{1}^{\text{fMet}}$ could have ring opened while exposed to neutral aqueous media during the rapid ion exchange column conversion of the tRNA to the lithium salt at room temperature and subsequent dialysis at 4° C. (No evidence by uv analysis for imidazole ring opening was found by exposing 7-methylguanosine in water for four days at 4° C.) Had this occurred, depurination during the HCl treatment is a possibility (Wintermeyer and Zachau, 1970).

Under the conditions of HCl exposure where the microscopic pH is 2.9, the possibility exists that adenine and

cytosine bases are protonated, leading to changes in the tertiary and/or secondary structures. The original conformational integrity of the tRNA is not obtained upon neutralization, nor is renaturation brought about by heating in the presence of Mg^{+2} . Aggregation is indicated in aqueous solutions of tRNA^{fMet}₁(HCl). It would seem that conclusions about the effect of acid catalyzed residue removal upon the conformation of specific regions or upon the overall conformation of a given tRNA should take into account the effect of acid per se.

ACKNOWLEDGMENTS We thank Drs. George P. Kreishman, Phoebe Dea and Paulus Kroon for several spectra and Professor Sunney Chan, California Institute of Technology, for use of the HR-220 spectrometer.

≠ MRC Laboratory of Molecular Biology, Cambridge, England

* Address correspondence to this author

REFERENCES

- Crawford, J. E., Chan, S.I. and Schweizer, M.P. (1971) Biochem. Biophys. Res. Comm. 44, 1-7
- Fasman, G.D., Lindblow, C. and Seaman, E. (1965) J. Mol. Biol. 12, 630-640
- Ghosh, K. and Ghosh, H.P. (1972) J. Biol. Chem. 247, 3369-3375
- Nakanishi, K., Furutachi, N., Funamizu, M., Grunberger, D., and Weinstein, I.B. (1970) J. Amer. Chem. Soc. 92, 7617-7619
- Pongs, O. and Reinwald, E. (1973) Biochem. Biophys. Res. Comm. 50, 357-363

- Reeves, R. H., Cantor, C.R. and Chambers, R.W. (1970)
Biochemistry 9, 3993-4001
- Schweizer, M.P., Chan, S.I. and Crawford, J.E. (1973) in
Physico-chemical Properties of Nucleic Acids
(Duquesne, J., ed) Vol 2, Academic Press, London
- Thiebe, R. and Zachau, H.G. (1968) Eur. J. Biochem. 5,
546-555
- Thiebe, R. and Zachau, H.G. (1970) Biochim. Biophys. Acta.
217, 294-304
- Wimmer, E., Maxwell, I.H. and Tener, G.M. (1968) Biochem-
istry 7, 2623-2628
- Wintermeyer, W. and Zachau, H. G. (1970) FEBS Letters 11,
160-164