SIGNIFICANCE AND MECHANISM OF DIVALENT-ION BINDING TO TRANSFER RNA

M. GUÉRON AND J. L. LEROY
Groupe de Biophysique, Ecole Polytechnique, 91128 Palaiseau Cédex, France

ABSTRACT Phosphorus NMR shows that divalent ions (manganese) bind to tRNA phosphates as to those of DNA or isolated phosphodiesters. The time for dissociation of a phosphate-divalent ion complex is in the microsecond range. For no single phosphate is the affinity to divalent ions >10 times that of the average phosphate. It is often stated that a small number of strong binding sites exist and are structurally and functionally important. This concept originates from binding curves whose properties should, instead, be traced to the polyelectrolyte nature of nucleic acids. The ^{31}P NMR data preclude the existence of strong sites to which divalent ions would bind very selectively. The spectroscopic and crystallographic observations of sites for divalent ions do not in fact demonstrate selective binding to these sites.

INTRODUCTION

It has long been thought that a small number of divalent ions, strongly bound at a small number of sites, play an important role in stabilizing the tertiary structure of tRNA (1, 2). Such sites have been proposed on the basis of the high affinity of tRNA for divalent ions and the curvature of the Scatchard binding plots (3–7). They have provided the framework for interpretations of proton NMR broadening by paramagnetic cations (8, 9) and energy transfer to rare earth metals (10, 11). They have been identified with intramolecular sites where magnesium is observed in tRNA crystals (12–15). Their possible role in the interaction of tRNA with synthetase or ribosome has been considered (15).

Divalent ions do bind to tRNA. But reflection and experiment suggest that the above picture, let us call it the canonical interpretation, is seriously wrong. The problem stems from a preconception based on enzyme physical chemistry. If an enzyme binds metal ions, and binding sites are found, and the ions are shown to be functionally required, then it is usually a sound presumption that one or a few ions located in as many key sites are essential structural or functional cofactors. This description applies typically to metalloenzymes such as carboxypeptidase or alkaline phosphatase, for example. On the other hand, because nucleic acids are highly charged molecules (polyelectrolytes) they will inevitably gather metal ions in high concentration near them. They also provide many weak binding sites (e.g., phosphates) with more or less differentiated properties depending on local structure (16–18). The conjunction of large cation concentration and numerous weak binding sites will superficially mimic strong binding. Furthermore, the reduction of interphosphate charge repulsion by ions will be a determinant of molecular conformation.

Thus, in the case of a neutral or weakly charged protein, evidence of metal binding and metal sites is a strong indication that one or some metal ions play an important role. But with nucleic acids it should be obvious from the start that metal ions will, in vast numbers, flock around them, bind, and influence their structure. The question to be asked, therefore, is not if tRNA binds metal ions, but whether there are, among the many binding sites, a small number of "strong sites," i.e., sites to which metal ions would go in strong preference to others, and whether such sites, if they exist, have a special structural role. As we shall show below, none of the measurements mentioned above provides unequivocal evidence for strong sites in tRNA. Further experiments, to which we now turn, have been carried out in the search for such sites; their results are dubious or negative.

^{31}P NMR Experiments

Our view is that metal ions interact with tRNA in much the same way as with regular, double-stranded polynucleotides such as DNA in the B form, and that the peculiarities of tRNA structure do not generate singular binding sites for divalent ions. This view is detailed by the study of the following questions, which aim at the characterization of the modes and sites of binding of Mn, a good substitute (19, 20) for the native divalent ion, Mg. (*a*) Does the first bound manganese bind to a unique site? (*b*) How does manganese bind to phosphates? (*c*) Are there other binding sites? (*d*) Could the first bound manganese ions spend a large part of their time in privileged sites?

The first question is answered in the negative by the observation (16) that manganese broadens the magnetic resonances of all tRNA phosphates (Fig. 1). Manganese must therefore come close to each phosphate. This excludes a unique binding site.

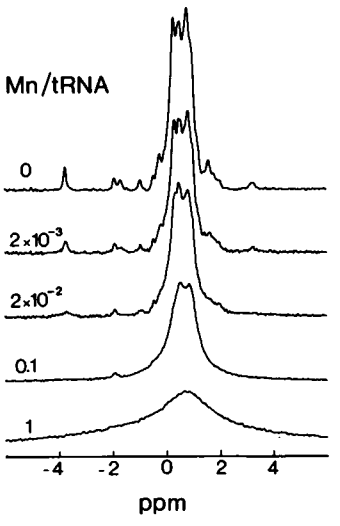

FIGURE 1 Broadening of 111.7 MHz ^{31}P spectrum of yeast $tRNA^{Phe}$ by manganese. All phosphates, whether resolved or in the central peak, are broadened, implying migration of the ion to the vicinity of each phosphate. The terminal phosphate is at −3.7 ppm. All spectra were processed identically, using a moderate line narrowing. Conditions are 1 mM tRNA, T = 30°C, 150 mM NaCl, 45 mM triethanolamine chloride, pH = 7.4, no magnesium. Broadening differentials between phosphates are reduced in the presence of magnesium.

The second question is addressed by first considering whether manganese moves uniformly among phosphates. The spectra in Fig. 1 show that different phosphates are broadened to a different extent. Among the resolved peaks the most broadened is at −3.7 ppm. This is the 5′-terminal (21), which is doubly charged in the conditions of Fig. 1. At lower pH, its broadening is reduced. Among the phosphodiesters, the one at 3.2 ppm is the most broadened, ~10 times more than those in the central, unresolved peak at 0.5 ppm. This does not result in good specificity, as it would imply (taking into account the longitudinal relaxation values) an occupancy of this site by the first bound manganese no larger than 10/76 or 13%.

The phosphate at −2 ppm is broadened 10 times less than average; like the other resolved peaks, its chemical shift is induced by the tertiary structure; it is not sensitive to magnesium. Those observations suggest that it may be somewhat inaccessible to ions, and they provide clues to its future identification.

Although the phosphates in the central peak are unresolved, they may be studied by spectrum comparison and subtraction (16): no phosphates are broadened as much as the one at 3.2 ppm. A few at most, and probably none, are as insensitive to manganese as the one at −2 ppm.

The NMR broadening can be shown to be in the slow exchange regime, so that the line width is equal to the average time interval between successive collisions of a given phosphate with manganese. From this, one derives a superior limit for the sticking time to the average phosphate, $\sim 3 \times 10^{-6}$ s at 30°C. This may be compared with the sticking time to AMP, $\sim 7 \times 10^{-7}$ s.

By contrast, phosphorus longitudinal relaxation is in the fast-exchange regime and can therefore be used to provide an average Mn-to-P distance (22). This is found to be 0.46 nm at 25°C. The distance decreases at higher temperature, suggesting an equilibrium between outer- and inner-sphere binding such as is observed for metal binding to isolated anions (23) (see below). In summary, it appears to us that manganese binds to tRNA phosphates as it does to a simple isolated phosphodiester, except for the enhanced concentration of manganese around the highly charged molecule.

The third question (are there binding sites other than phosphates?) cannot be answered on the basis of the ^{31}P NMR data quoted above. For instance, if manganese were bound to phosphate as an inner-sphere complex only, at a distance of 0.3 nm, 8% of the time, and bound in other locations 92% of the time, the average distance measured by longitudinal relaxation would be $(0.08)^{-1/6} \times 0.3 = 0.46$ nm, as found.

Such hypothetical sites could be of two different types. The first type is exemplified by ribose hydroxyl groups, as seen for instance by x-ray crystallography in the case of sodium binding to adenylyl-(3′,5′)-uridine ApU) (24). Because there is one ribose for each phosphate, these sites are quite numerous. Binding to the base may also occur. These matters could be investigated in principle by proton or ^{13}C NMR. Whatever the answer, they bear little on the concepts addressed here.

The second type of site to be considered is a single strong site. If the binding of Mn 8% of the time is enough to explain the relaxation of ^{31}P by Mn, might not Mn be trapped in one strong site the remaining 92% of the time? This fourth question is answered in the negative by the measurements of Figs. 2 and 3.

In Fig. 2 the relaxation rates are shown to be proportional to manganese concentration. In these experiments, phosphorus relaxation is used as a probe of the available manganese. If the first manganese ions were trapped in a strong site most of the time, they would relax the average phosphate poorly; ions added afterwards would relax the average phosphate more, because the strong sites would then be saturated. This is not observed.

In Fig. 3, the average Mn-to-P distance is derived from the longitudinal relaxation of ^{31}P, for different polynucleotides. There are variations with temperature, and differences between species. However the results for DNA and tRNA are very close. This result would be hard to explain if manganese bound to tRNA were sequestered in one or a few strong sites. Relaxation of ^{31}P by cobalt in tRNA, DNA, and polyI-polyC supports the present analysis, as will be shown elsewhere.

OTHER OBSERVATIONS

In the previous section, we summarized the evidence from ^{31}P NMR that divalent ions bind to tRNA much as they do to other double-stranded polynucleotides. We now proceed

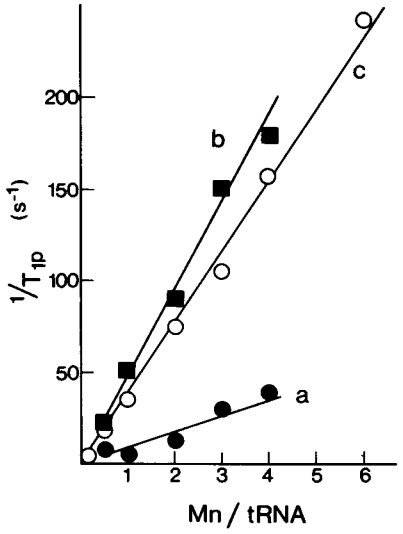

FIGURE 2 Longitudinal relaxation of phosphates induced by manganese. In the absence of magnesium, 18 ± 5 phosphates (*a*) are relaxed six times less than the 58 others (*b*). (Isolated phosphates are not discernable due to line broadening at the high concentrations of manganese used.) With 20 Mg/tRNA, relaxation is uniform for all phosphates (*c*). The slope of line *c* is that expected under the assumption that addition of magnesium causes only a redistribution of manganese among phosphates. This is ascribed to the diminution of the electrostatic potential and therefore of its spatial variations, by the large number of bound divalent ions. The redistribution does not imply the suppression of trapping sites by magnesium competition. In each case (*a*, *b*, or *c*) relaxation is proportional to the number of manganese per tRNA (all are bound at the concentrations used). This excludes the possibility that the first bound ions would be trapped in a small number of sites. Conditions are 1 mM yeast $tRNA^{Phe}$, T = 5°C, 150 mM NaCl, 45 mM triethanolamine chloride, pH = 7.4.

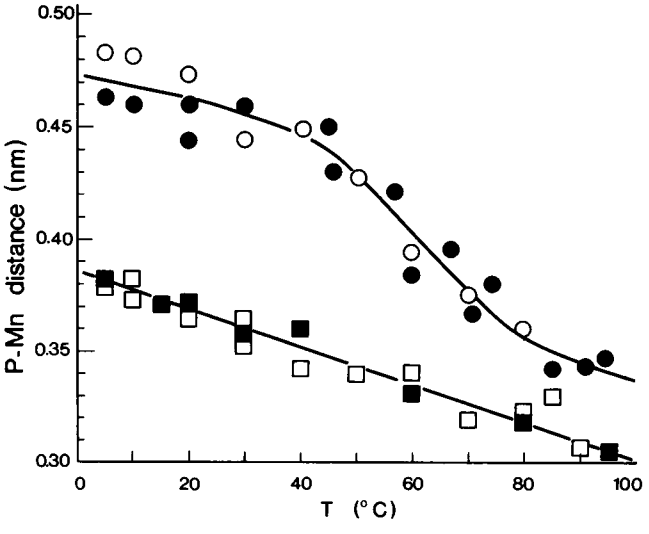

FIGURE 3 The average distance $\langle (r^{-6})^{1/6} \rangle$ between phosphorus and manganese is derived from the longitudinal relaxation data: tRNA (●); DNA, 30 ± 5 bp, doubled-stranded, devoid of nicks or single-stranded ends, enzymatically prepared[37] (○); single-stranded polyadenylate either 150 nucleotides long, or longer and uncontrolled (■); single-stranded polyuridylate (□). (The correlation time was determined at each temperature by performing measurements at two frequencies, 111.7 and 40.5 MHz.) The similarity of DNA and tRNA values argues against a small number of strong sites on tRNA, independently of the complex features of the data. These are the temperature-dependence that may indicate an outer-sphere to inner-sphere equilibrium, and the difference between double- and single-stranded polynucleotides (this could involve bridging of two phosphates by the divalent ion in the case of the more flexible, single-stranded, species).

to show that all other published data can be accommodated within this conceptual framework.

(*a*) The curvature of the Scatchard plots and the large affinity of tRNA for divalent ions are easily understood on the basis of the large charge of the molecule. The concentration of counterions is strongly increased in the vicinity of the polyelectrolyte (25), and binding (e.g., to phosphates) is thereby enhanced. Binding curves in agreement with observations can be computed even in the oversimplified description of uniform binding to all phosphates (17) with a binding constant (26) equal to that of Mn for adenylyl-(3',5')-adenosine (ApA) $\sim 10(\text{M/liter})^{-1}$. The effect of ionic strength is accounted for quantitatively. The interpretation is bolstered by the observation that binding to tRNA and DNA (27) are quite similar. Although widespread, the inference of two classes of sites, strong and weak, on the basis of the Scatchard plots is therefore mistaken.

The binding curve of Mg to *Escherichia coli* $tRNA^{fMet}$ deserves further notice. This was fitted to one site 70 times stronger than 26 others (3). We think that this result should be viewed with caution. The experiment is difficult. It was carried out at 4°C and may not be representative of the situation at 37°C (17). Polyelectrolyte effects are ignored in the interpretation, and this raises questions even though such effects may be reduced at the high salt concentrations used (0.17 M). For instance, it is clear that the total number of sites must be at least 76 (1 per phosphate) and not 27. There may also be differences between the different tRNA, but we are not prepared at this time to assume radical differences in the type or significance of divalent ion binding in the case of one tRNA species.

One often hears that it is difficult to avoid contamination of tRNA by polyvalent ions. This is not evidence for strong binding sites. The situation arises because tRNA is a polyelectrolyte. If this is taken into consideration, the cleaning of tRNA becomes a simple matter, as discussed in the Appendix.

(*b*) Local probes are provided by spectroscopic methods such as NMR or luminescence transfer or quenching. They report on the presence of divalent ions in a given locus, but not on its absence elsewhere. The occupancy of the probed locus and the kinematics of motion to and from the locus can only be derived from a quantitative analysis, and then not always. Such analysis is usually not provided in earlier work.

Thus, it has been shown that the NMR peaks of some exchangeable interbase protons are selectively broadened by cobalt (8). Do these protons signal special binding sites? Not necessarily. Selective proton broadening is expected even if cobalt binds only to phosphates, identically to all of

them. For then only the few protons close to phosphates may be broadened.

This interpretation is supported by the comparison of proton and phosphate broadening by cobalt. It is also in reasonable agreement with phosphorus-proton distances derived from the crystal structure (28) together with NMR assignments (8). (This should however be viewed with caution, as NMR and crystal structure refer to two different tRNA species in this case.)

Consider now luminescence experiments. Fluorescence enhancement of the *Y* base by magnesium has been extensively studied (29). Together with 4-thiouracil (s^4U) quenching by manganese (30), it shows that divalent ions favor the central region of tRNA in low salt. Even among chemically identical binding sites for ions, those in the central region of tRNA are expected to show larger affinity due to the large electrostatic potential induced by the large phosphate concentration in this area. Such effects are expected and found to diminish in high salt. Furthermore, not only magnesium but sodium also enhances *Y*-base fluorescence.

Energy transfer to rare earth metals (10, 11) from s^4U has been interpreted in terms of strong sites. Beyond the general comments that have been made above regarding local probes, one should remember that the experiments used trivalent terbium and europium. The affinity differences due to the regional electrostatic potential should be higher for trivalent than for divalent ions, particularly in low salt concentration (25).

(*c*) X-ray crystal structures of yeast $tRNA^{Phe}$ show a number of sites harboring divalent metal ions. The latest publications concerning bound metals in the monoclinic (14) and orthorhombic (15) crystals present similar features, although there are differences. These may be real (crystal effects) or may reflect artifacts of the refinement procedures. A large variety of sites have been described. Divalent ions coordinate to phosphate, ribose, and base ligands, mostly through water. Direct coordination of magnesium occurs with phosphate only. Multiple direct coordination is seen in one case, to P20 and P21. A second case, to P14 and P57, is found for the monoclinic, but not for the orthorhombic crystal.

One direct coordination is found to P19. The interior of the anticodon loop harbors Mg coordinated directly to P37 in the orthorhombic crystal, but only water is found in the monoclinic crystal. The same difference exists regarding the existence of a Mg directly coordinated to P2. One Mg binds exclusively through water, to P8, P9, P11, and P12. This is observed in both studies. Another Mg is located in the deep groove of the TΨC helix (orthorhombic only).

One may note that the Mg coordinated to P20 and P21, and the one coordinated to P19 are coordinated also, through water, to ligands of another tRNA.

Jack et al. (13) observed that upon partial substitution of magnesium by manganese, the Mg bound to P19 is expelled. A manganese ion binds 0.2 nm away from the magnesium site, with direct coordination to base nitrogen N7 of G20.

Besides the magnesium ions described above, the crystal must contain others, for a total of ~34 per tRNA, because magnesium is the only counterion apart from two spermines per tRNA. Faint electron density peaks, found near phosphate or ribose oxygens, have indeed been ascribed to a large number of magnesium ions (13) although this interpretation is uncertain (14). The lower density may be due to a more variable binding geometry, which would be expected of ions bound on the outside of the molecule: "A cation which could occupy two different coordination geometries with the same likelihood would appear as averaged electron density peak and probably be close to the solvent or background electron density" (15).

However, one should be careful to note that sites in which the ions are more mobile do not *ipso facto* have a lower affinity than those where ions are less mobile and hence better seen. In fact, relative affinity of different sites cannot be measured from properties of a crystal of the magnesium salt: monovalent counterions, with only a few magnesium per tRNA added, would be needed. Thus the x-ray data do not provide evidence for thermodynamically strong sites. Rather, they display best the less mobile of a long series of bound ions.

Among the magnesium ions found in the orthorhombic crystal, the one that coordinates directly to P2 also coordinates through water to P1. Because P1 is identified in the NMR spectrum (at −3.7 ppm), a direct comparison can be made. Using the same arguments as above for the phosphate at 3.2 ppm, the P1-P2 site is occupied at most 15% of the time by the first manganese bound to tRNA in solution.

Comparison can also be made between the crystallographic and the binding results. The x-ray data are typically collected at low temperature (4°–8°C). In this range of temperature, the binding of magnesium to *E. coli* $tRNA^{fMet}$ was studied and interpreted in terms of one strong site. One may contrast this interpretation with that of the crystal structure in terms of multiple strong sites. We question both of these interpretations.

The variety of binding modes, the extent of outer-sphere binding, the rarity of multiple direct coordination, the imprecision of the manganese substitution, all argue against attempting to categorize the crystallographic sites by affinity for divalent ions.

(*d*) Lastly, it may be asked whether a case for strong sites can be made on the basis of some functional property, such as formation of the native structure or capability of sustaining a reaction like aminoacylation by a synthetase. Here again the answer is negative. The tertiary structure is formed even in the absence of divalent ions, as shown, for example, by the low-field NMR of exchangeable protons (31) in 150 mM NaCl. It is true that some changes are induced by divalent ions, as shown by proton (31) and phosphorus NMR (21, 32, 33) and by light scattering

(34). Their significance or extent are not known at present. Most important, they occur not for the first magnesium ion bound, but on the contrary at high concentrations, such as 1 mM Mg. At such a concentration, bound ions would number ~10 (in 150 mM NaCl) (16).

Concerning a requirement of divalent ions for aminoacylation, its study is complicated by the involvement of ATP-bound metal in the formation of aminoacyl adenylate. This problem should therefore be examined in systems where the adenylate has been formed beforehand.

There is, however, conflicting evidence concerning the role of divalent ions in tRNA aminoacylation (35). Divalent cations may be required by some species but not others, or again at pH 7 but not at pH 6.2 (36), and the buffer itself could be involved. The ions might affect tRNA structure, tRNA-enzyme interaction, or catalysis of aminoacyl transfer. In its present state, this important question provides no information on our subject.

CONCLUSION

In conclusion, the electric charge of tRNA is responsible for its large affinity to cations in general and divalent cations in particular. Correlatively, cations may influence the shape of the molecule. These properties belong not to tRNA alone, but to other nucleic acids as well.

The irregular shape and charge distribution of tRNA will be reflected to some extent in the distribution of ions, the central region being favored. But this does not entail the existence of strong sites exhibiting an original type or organization of chemical ligands. There is, in fact, no evidence for strong sites of this or other types. It appears that binding is mainly to phosphates and that it is comparable, kinetically and thermodynamically, to binding to model monomers, when account is taken of the increased concentration around a polyelectrolyte.

It is only slightly oversimplified to claim that divalent ions bind to tRNA as they bind to DNA, and that they bind to DNA as they do to dinucleotides.

APPENDIX

Note on the Preparation of tRNA Devoid of Divalent Metal Ions

It is often considered that tRNA is difficult to clean of divalent ions. Procedures typically involve extensive dialysis against buffer containing EDTA or with added Chelex beads. Sometimes the tRNA is heated up to 60°C, with the goal of destroying specific structures that would be involved in ion binding.

Because tRNA is a polyelectrolyte, its apparent affinity for polyvalent ions in a solution containing monovalent ions depends critically on ionic strength. At low ionic strength, it is difficult to eliminate divalent ions, or even to maintain the purity of a tRNA solution. However, cleaning and conservation of tRNA is easily achieved at high ionic strength, for instance in 100 mM NaCl. Analysis by spark emission and by atomic absorption shows that extraneous metals can easily be reduced to <0.1 ion tRNA (17).

Presently, we use a one-step purification procedure in which a tRNA solution (100 mM NaCl, 40 mM triethanolamine buffer, pH 7.4) is passed through a 10-cm high Chelex column at room temperature. After this simple operation the concentration of extraneous metals is nil, i.e., <0.1 ion/tRNA, as judged by atomic absorption analysis for Mg and Ca.

Received for publication 17 September 1981 and in revised form 13 November 1981.

REFERENCES

1. Crothers, D. M., and P. E. Cole. 1978. Conformational changes of tRNA. *In* Transfer RNA. S. Altman, editor. MIT Press. 196–247 (and references cited therein).
2. Schimmel, P. R., and A. G. Redfield. 1980. Transfer RNA in solution: selected topics. *Annu. Rev. Biophys. Bioeng.* 9:181–221 (and references cited therein).
3. Stein, A., and D. M. Crothers. 1976. Equilibrium binding of magnesium (II) by *E. coli* $tRNA^{fMet}$. *Biochemistry.* 15:157–160.
4. Römer, R., and R. Hach. 1975. tRNA conformation and magnesium binding. A study of yeast phenylalanine-specific tRNA by a fluorescent indicator and differential melting curves. *Eur. J. Biochem.* 55:271–284.
5. Schreier, A. A., and P. R. Schimmel. 1974. Interaction of manganese with fragments, complementary fragments recombinations, and whole molecules of yeast phenylalanine specific transfer RNA. *J. Mol. Biol.* 86:601–620.
6. Danchin, A., and M. Guéron. 1970. Cooperative binding of manganese (II) to transfer RNA. *Eur. J. Biochem.* 16:532–536.
7. Cohn, M., A. Danchin, and M. Grunberg-Manago. 1969. Proton magnetic relaxation studies of manganous complexes of transfer RNA and related compounds. *J. Mol. Biol.* 39:199–217.
8. Hurd, R. E., E. Azhderian, and B. R. Reid. 1979. Paramagnetic ion effects on the nuclear magnetic resonance spectrum of transfer ribonucleic acid: assignment of the 15-48 tertiary resonance. *Biochemistry.* 18:4012–4017.
9. Chao, Y. H., and D. R. Kearns. 1977. Manganese (II) as a paramagnetic probe of the tertiary structure of transfer RNA. *Biochim. Biophys. Acta.* 477:20–27.
10. Kayne, M. S., and M. Cohn. 1974. Enhancement of Tb(III) and Eu(III) fluorescence in complexes with *E. coli* tRNA. *Biochemistry.* 13:4159–4165.
11. Wolfson, J. M., and D. R. Kearns. 1974. Europium as a fluorescent probe of metal binding sites on transfer ribonucleic acid. I. Binding to *E. coli* formylmethionine transfer ribonucleic acid. *J. Am. Chem. Soc.* 96:3653–3654.
12. Quigley, G. J., M. M. Teeter, and A. Rich. 1978. Structural analysis of spermine and magnesium ion binding to yeast phenylalanine transfer RNA. *Proc. Natl. Acad. Sci. U.S.A.* 75:64–68.
13. Jack, A., J. E. Ladner, D. Rhodes, R. S. Brown, and A. Klug. 1977. A crystallographic study of metal-binding to yeast phenylalanine transfer RNA. *J. Mol. Biol.* 111:315–328.
14. Hingerty, B., R. S. Brown, and A. Jack. 1978. Further refinement of the structure of yeast $tRNA^{Phe}$. *J. Mol. Biol.* 124:523–534.
15. Teeter, M. M., G. J. Quigley, and A. Rich. 1980. Metal ions and transfer RNA. *In* Metal Ions in Biology. Nucleic Acid-Metal Ion Interactions. T. G. Spiro, editor. John Wiley & Sons, Inc., New York. 1:145–177.
16. Guéron, M., and J. L. Leroy. 1979. The binding of divalent ions to transfer nucleic acids. *In* ESR and NMR of Paramagnetic Species in Biological and Related Systems. I. Bertini and R. S. Drago, editors. Reidel Publishing Co., Dordrecht, Holland. 327–367.
17. Leroy, J. L., and M. Guéron. 1977. Electrostatic effects in divalent ion binding to tRNA. *Biopolymers.* 16:2429–2446.
18. Walters, J. A. L. I., H. A. M. Geerdes, and C. W. Hilbers. 1977. On the binding of Mg^{2+} and Mn^{2+} to tRNA. *Biophys. Chem.* 7:147–151.
19. Danchin, A., and M. Guéron. 1970. Proton magnetic relaxation

study of the manganese-transfer-RNA complex. *J. Chem. Phys.* 53:3599–3609.

20. Hyafil, F., and S. Blanquet. 1977. Methionyl-tRNA synthetase from *E. coli* fJ1: substituting magnesium by manganese in the L-methionine activating reaction. *Eur. J. Biochem.* 74:481–493.
21. Guéron, M., and R. G. Shulman. 1975. ^{31}P magnetic resonance of tRNA. *Proc. Natl. Acad. Sci. U.S.A.* 72:3482–3485.
22. Shulman, R. G., H. Sternlicht, and B. J. Wyluda. 1965. Study of metal-ion binding to nucleic acids by ^{31}P nuclear magnetic resonance. *J. Chem. Phys.* 43:3116–3122.
23. Peguy, A., and H. Diebler. 1977. Kinetics and equilibria of the binding of cobalt (II) to adenosine 5'-monophosphate. *J. Phys. Chem.* 81:1355–1358.
24. Seeman, N. C., J. M. Rosenberg, F. L. Suddath, J. J. P. Kim, and A. J. Rich. 1976. RNA double-helical fragments at atomic resolution: the crystal and molecular structure of sodium adenyl-3',5'-uridine hexahydrate. *J. Mol. Biol.* 104:109–144.
25. Weisbuch, G., and M. Guéron. 1981. Polyelectrolyte theory. II. Activity coefficients in Poisson-Boltzmann and in condensation theory. The polarizability of the counterion sheath. *J. Phys. Chem.* 85:517–525.
26. Koren, R., and A. S. Mildvan. 1971. Magnetic resonance and kinetic studies of the role of the divalent cation activator of RNA polymerase from *E. coli*. *Biochemistry.* 16:241–249.
27. Reuben, J., and E. J. Gabbay. 1975. Binding of manganese (II) to DNA and the competitive effects of metal ions and organic cations. An electron paramagnetic resonance study. *Biochemistry.* 14:1230–1235.
28. Ladner, J. E., A. Jack, J. D. Robertus, R. S. Brown, D. Rhodes, B. F. C. Clark, and A. Klug. 1975. Atomic co-ordinates for yeast phenylalanine tRNA. *Nucleic Acids Res.* 2:1629–1637.
29. Ehrlich, R., J. F. Lefèvre, and P. Rémy. 1980. Fluorimetric study of the complex between yeast phenylalanyl-tRNA synthetase and $tRNA^{Phe}$. *Eur. J. Biochem.* 103:145–153.
30. Leroy, J. L., M. Guéron, G. Thomas, and A. Favre. 1977. Role of divalent ions in folding of tRNA. *Eur. J. Biochem.* 74:567–574.
31. Johnston, P. D., and A. G. Redfield. 1981. Nuclear magnetic resonance and nuclear Overhauser effect study of yeast phenylalanine transfer ribonucleic acid imino protons. *Biochemistry.* 20:1147–1156.
32. Muller, A., M. Guéron, and J. L. Leroy. 1980. The binding of divalent ions to tRNA. *EMBO (European Molecular Biology Organization)/FEBS (Fed. Eur. Biochem. Soc.) tRNA Workshop. (Abstr.)* 16–21.
33. Salemink, P. J. M., E. J. Reijerse, L. C. P. J. Mollevanger, and C. W. Hilbers. 1981. Conformational changes of yeast $tRNA^{Phe}$ as monitored by ^{31}P NMR. *Eur. J. Biochem.* 115:635–641.
34. Potts, R., M. J. Fournier, and N. C. Ford. 1977. Effect of aminoacylation on the conformation of yeast phenylalanine tRNA. *Nature (Lond.)* 268:563–564.
35. Lövgren, T. N. E., A. Petersson, and R. B. Loftfield. 1978. The mechanism of aminoacylation of transfer ribonucleic acid. The role of magnesium and spermine in the synthesis of isoleucyl-tRNA. *J. Biol. Chem.* 253:6702–6710.
36. Zimmerman, T. P., and B. Robison. 1972. Effect of assay conditions on the magnesium requirement of the transfer reaction catalyzed by phenylalanyl-tRNA synthetase from baker's yeast. *Biochem. Biophys. Res. Commun.* 47:1138–1143.
37. Early, T. A., and D. R. Kearns. 1979. ^{1}H nuclear magnetic resonance investigation of flexibility in DNA. *Proc. Natl. Acad. Sci. U.S.A.* 76:4165–4169.