SALT INDUCED DEPROTONATION OF INITIALLY SALT-FREE TRANSFER RNA

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SUMMARY

Transfer RNA, initially salt-free, releases protons upon treatment with magnesium or sodium chloride, and simultaneously an "ionic" structure of tRNA is changed into the normal base-stacked one. The number of released protons was measured by the uptake of NaOH necessary for maintaining constant pH in tRNA solutions during their treatment with salts. At pH 4.5 about 20 protons were released per tRNA molecule; at higher pH less protons were released and at pH 7.5 no Mg^{2+} effected deprotonation of tRNA took place.

INTRODUCTION

Divalent cation interactions with transfer RNA have been investigated by many authors (1-4,7-14), and though the experimental data show good overall agreement, there remain discrepancies in their interpretations. Some authors suggest that tRNA binds Mg^{2+} in a cooperative way (1,2), others consider a sequential process with two (or more) types of independent binding sites involved (3). Although most of the authors observed Mg^{2+} induced conformational transitions of tRNA molecules, the native conformation being achieved at about 5 mM Mg^{2+}, recently the existence of native tertiary structure was demonstrated for Mg^{2+}-free $tRNA^{Phe}$ (4). The tertiary structure of the Mg^{2+}-free $tRNA^{Phe}$ melts below room temperature, which has been suggested to explain why in the earlier studies done at room temperature tRNA was susceptible to Mg^{2+} effected conformational changes. Conformation of tRNA depends, however, not only on temperature or cation concentration but also on pH of the solvent; bearing this in mind, the action of each of these individual factors should be correlated. Although appropriate investigations of DNA (5) and of synthetic polyribonucleotides (6) have shown

strong effects of salts on apparent pK values for protonation of their bases, surprisingly little attention has been devoted to the dependence of Mg^{2+}-tRNA structure relations upon pH. Only recently Lynch and Schimmel (7) demonstrated that Mg^{2+} binding to $tRNA^{Ile}$ was strongly pH dependent due to the abnormally high base pKs of tRNA in low-salt, Mg^{2+}-free solutions. Bina-Stein and Crothers showed pH dependent conformational changes of Mg^{2+}-free $tRNA^{Tyr}$ with a greater proton affinity to its acid form than to its neutral one (8). Effect of pH on Mg^{2+} induced fluorescence changes of $tRNA^{Phe}$ from barley was also observed (9), as well as NaCl dependent changes of pH in unbuffered, concentrated solutions of tRNA (10,11).

In the present paper evidence is shown that the protonation of tRNA (at slightly acidic pH) strongly depends upon salt concentration of the solvent; that protonated salt-free tRNA releases protons when treated with $MgCl_2$ or NaCl, and that the number of released protons depends on the pH of the initial salt-free tRNA solution.

MATERIALS AND METHODS

Yeast transfer RNA (Na, K salt) was obtained from Boehringer (Mannheim, GFR), dialysis tubing from Union Carbide Corp. (USA), other chemicals were obtained from Polskie Odczynniki Chemiczne (Gliwice, Poland).

Titrations and pH measurements were done using a PHM-26 pH-meter equipped with autoburette ABU-12, titrator TTT-11, and SBR-2 recorder (Radiometer, Denmark).

The salt-free tRNA solution was prepared as follows: about 200 mg of tRNA were dissolved in 20 ml of 0.02 M EDTA·2Na and dialysed at 4°C against two changes (24 hours each) of the same solvent, followed by five changes (24 hours the first two, 12 hours the remainders) of water double-distilled from quartz. After dialysis the solution was either diluted with water or lyophilised in order to obtain tRNA concentrations in the range of 20 to 120 A_{260} units per ml. The solution was stored at -18°C.

For titration-curve recording, 5 ml of tRNA solution (60 A_{260} units per ml, salt free, containing $MgCl_2$ or NaCl) were placed in a thermostated (20°C) cell and automatically titrated, using ABU-12 with a 2 ml syringe.

The number of protons released from tRNA by Mg^{2+} or Na^+ was determined in the following way: 5 ml of the salt-free tRNA solution (10 - 120 A_{260} per ml) were placed in a thermostated pH-meter cell and adjusted with either NaOH or HCl to any preselected pH value within range 4.5 - 6.5, or pH of the sample was left unchanged after dialysis (4.5 - 5.2, depending on tRNA concentration). The pH-meter was then switched to pH-stat and small portions (usually 5 μl) of $MgCl_2$ (0.01 - 1.0 M adjusted to pH as tRNA) or 1.0 M NaCl were added to the tRNA solution. pH Decrease of the solution was observed after each salt addition, then the pH was brought back to the initial value with standardised NaOH delivered from the pH-stat. A single portion of salt introduced to the tRNA solution was chosen so as to effect a pH drop of not greater than 0.35. When calculating results, it was assumed that the average molecular weight of yeast tRNA is 26500 daltons and that 1 mg of tRNA is equivalent to 21.5 A_{260} units (12).

RESULTS

Hydrogen ion titration curves of tRNA in the presence and absence of salts are shown in fig.1. The final (pH 8) uptake of alkali by the Na^+-containing, and by salt-free tRNA solutions were the same, while the Mg^{2+}-containing sample gave a slightly higher result. However, in the presence of Mg^{2+} or Na^+, a significant number of protons were titratable at a much lower pH than in the case of the salt-free tRNA. The difference in NaOH uptake (at pH below 8) by solutions containing Mg^{2+} or Na^+ and by the salt-free tRNA samples suggests, that under the influence of cations, salt-free tRNA releases protons.

The effect of Mg^{2+} on pH of the initially salt-free tRNA solution is shown in fig.2. Magnesium cations at concentrations up to about 10^{-3} M had little influence on the pH of the solution; however, increase of Mg^{2+} from 10^{-3} to 10^{-2} M brought about a rapid decrease in pH. Further Mg^{2+} additions resulted again in only small pH changes. (Note that in fig.2 there is a logarythmic cale of $[Mg^{2+}]$). Addition of $MgCl_2$ to more than $10^{-1.3}$ M caused precipitation of tRNA, accompanied by further increase of $[H^+]$.

The number of protons released from tRNA by Mg^{2+} (Na^+) at pH 4.5 - 6.5

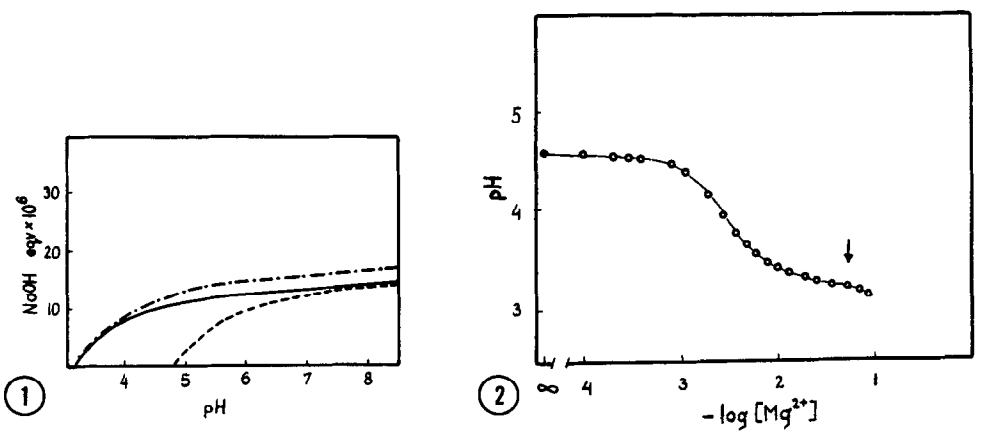

Figure 1

Hydrogen ion titration curves of salt-free tRNA (-----), and of the same solution adjusted either to 1 M NaCl (———) or to 0.01 M $MgCl_2$ (-.-.-.-.). Each titrated sample contained 300 A_{260} units of tRNA.

Figure 2

Changes of pH in the initially salt-free tRNA (60 A_{260} units per ml) solution upon addition of $MgCl_2$. Precipitation of tRNA marked with the arrow.

is shown in fig.3 to be a function of the cation/tRNA molar concentration ratio. At each investigated pH value, protons were released from tRNA rapidly until the Mg^{2+}/tRNA ratio reached a value of about 30. Further increase of $[Mg^{2+}]$ caused smaller increments of alkali uptake until finally, at Mg^{2+}/tRNA ratios above 10^3, a plateau was reached. The maximal number (20 ± 2) of protons released from tRNA at this saturating Mg^{2+} concentration was observed for tRNA solutions at "natural" pH (4.5 - 5.2).At higher pH values, under the influence of Mg^{2+}, less H^+ were released from tRNA and as shown in fig.4, the number of protons released per one tRNA molecule was a linear function of pH. Its extrapolation to higher pH values indicated that no protons would be released at pH above 7.5, which is in good agreement with the titration curves shown in fig.1.

The Mg^{2+}-induced dissociation of protons from tRNA was acompanied by a parallel decrease in UV absorbancy of the sample. The respective curve shown in fig.3 by a dotted line was measured at "natural" pH for a solution containing 12 A_{260} units of tRNA/ml. The measurements were carried

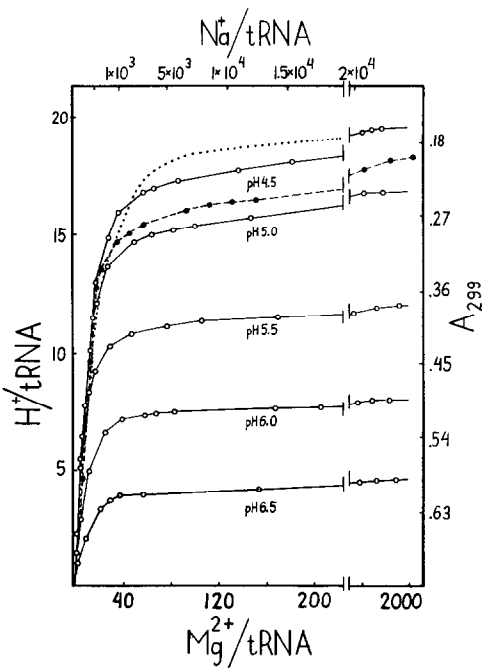

Figure 3

Effect of Mg^{2+} (———) and of Na^+ (----) on the number of protons released from tRNA. Mg^{2+}-effected absorbancy changes of tRNA at pH 4.5 (•••••).

out at 299 nm and the readings were taken after each $MgCl_2$ and respective NaOH additions. When less concentrated tRNA solutions were used for an experiment, hypochromicity could be measured at shorter wave-lengths and although absorbancy decrease was less drastic than that observed at 299 nm, in every case the absorbancy change curves were parallel to the curves of alkali uptake.

From the curves shown in fig.3, it was calculated that independent of pH, half of the Mg^{2+}-releasable protons were released from tRNA at 12 ± 2 Mg^{2+}/tRNA ratio. When Mg^{2+} concentrations respective for this ratio at various tRNA concentrations were plotted versus [tRNA], a straight line was obtained. Its extrapolation to [tRNA] = 0 gave value about 10^{-5} M $MgCl_2$, the reciprocal of which can be taken as an apparent average constant for Mg^{2+} binding to the tRNA sites responsible for release (or binding) of protons.

The measurements of protons dissociated from tRNA under the influence of Mg^{2+} were done at various pH. Higher than "natural" pH values of the starting tRNA solutions were obtained by apropriate adjustments with NaOH. When the number of the released protons was calculated from the sum of

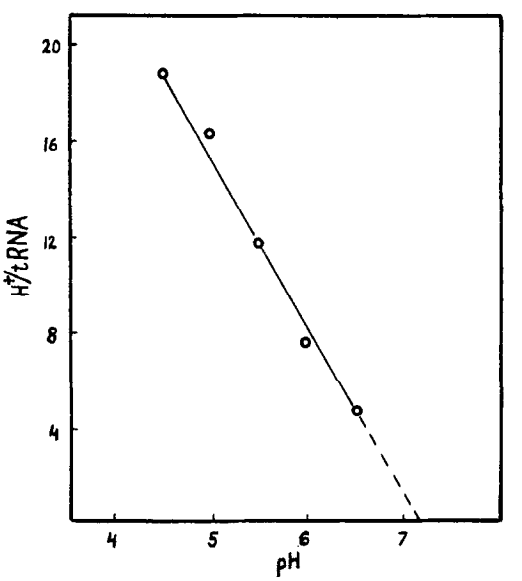

Figure 4

Influence of pH on the number of protons released from tRNA at saturating concentrations of Mg^{2+}.

alkali equivalents used both for the necessary pH adjustments and for the titrations, in each case 20± 2 protons per tRNA molecule were obtained, independent of pH or tRNA concentration.

It must be stressed, however, that the above number of protons was measured only when Mg^{2+} was introduced to the investigated sample in small protions followed by additions of NaOH necessary to maintain constant pH. When Mg^{2+} was added to tRNA in one portion in order to achieve a "saturating" concentration in one step, tRNA precipitated and the number of released protons was about 50% higher than observed during the normal procedure. No immediate precipitation was observed upon addition of the saturating amount of NaCl.

DISUSSION

Experiments reported in this paper provide direct evidence of the cation induced deprotonation of initially salt-free (and apparently protonated) tRNA, a phenomenon that hitherto has not been described because cation--tRNA interactions have always been investigated in buffered systems and at relatively low tRNA concentrations. As shown by NaOH titrations, under the influence of salts each tRNA molecule in an unbuffered aqueous solution releases about 20 protons. This means that in salt-free solution at least 25% of all tRNA bases are protonated and at this magnitude of protonation the obvious tendency would be to form ionic linkages between the positively charged bases and the negative phosphate groups. Removing of metal cations from tRNA by dialysis is rather a slow and gradual process, therefore it seems reasonable to assume that the respective base protonation also proceeds gradually, thus allowing for formation of ionic linkages mainly between those groups that were close to each other in the native, salt-containing tRNA structure. We suggest that during the desalting procedure a considerable proportion of ordered and stacking-stabilised regions of tRNA are converted into another ordered structure, stabilised by ionic interactions (8). The space necessary to bring the phosphate groups of one of the double helical strains into contact with bases of the other strain (and vice versa) could be provided for by the stretching of the native helix due to electrostatic repulsion between the unscreened phosphates.

The existence of salt-free tRNA structure, stabilised by "abnormal" base ionisation was already suggested by Lynch and Schimmel (7). Further, Bina--Stein and Crothers (8) demonstrated an acid tRNA from, almost as stable as the native one.

The deprotonation paralleled transition of tRNA structure, stabilised by base-phosphate ionic interactions, into the native structure of tRNA, could be explained as follows: Magnesium ions, introduced to the salt-free tRNA, compete with the protonated bases for phosphates, and bearing stronger charge, win this competition. The protonated bases are now allowed to dissociate according to their normal pKs, but as the unprotonated forms are trapped into base-pairing interactions, the equilibrium of base protonation is disturbed and the protons, initially base-bound, are released. At pH higher than "natural" for the salt-free tRNA solution, less bases would be protonated and less protons could be released by Mg^{2+}. At pH 7.5 (or higher, see fig.4) no protonation would take place at these bases, from which at lower pH protons would be released by magnesium cations.

The Mg^{2+}-effected conversion of ionic tRNA structure into the base-paired one is evidenced by the absorbancy changes closely parallel (at pH 4.5) to the respective curve of proton release (see fig.3). It is, however, not clear whether binding of Mg^{2+} to salt-free tRNA effects its direct convertion into the native structure, or whether sore intermediate forms are involved. Results obtained by Bina-Stein and Crothers (8) as well as those by Lynch and Schimmel (2,7) and others (13) suggest the latter possibility, however, the recent findings of Römer and Hach (4) are in favour of the former.

The release of more than 20 protons per single tRNA molecule observed upon precipitation of tRNA by way of addition of a single saturating amount of $MgCl_2$ suggests that in tRNA aggregates there are more base-pairs than in its native structure, which is consistent with observations published previously (14).

It should be pointed out that once Mg^{2+} is bound to a given tRNA site, the binding site changes its nature. Some change in the binding site nature should be likewise expected when Mg^{2+} is bound to possible intermediate tRNA forms. Depending thus on the initial state of tRNA used for investigation and on the probe used as binding indicator, one can obtain different Mg^{2+} binding constants. In the present investigation an apparent average constant of about 10^4-10^5 M was obtained for the Mg^{2+} binding to salt-free tRNA. This is in fairly good agreement with some previously published data (1,2).

The salt effected deprotonation of tRNA described in this paper obviously had to be preceded by its protonation, and in every case a change in tRNA conformation took place. Similar conformational changes, induced by a possible protonation (or deprotonation) of tRNA during its interaction with proteins, can play an important role in the various biological functions of these molecules.

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