Acknowledgments

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[3] Structure and Distance Determination in RNA with Copper Phenanthroline Probing

By THOMAS HERMANN and HERMANN HEUMANN

Introduction

Complexes of redox-active metals serve as chemical nucleases for probing secondary and tertiary structure of RNA molecules.[1–4] Among these metal complexes, 1,10-phenanthroline-copper (OP-Cu) is especially useful, as it cleaves RNA with high specificity in ordered single-stranded regions.[5–8] The cleavage reaction proceeds in an oxidative attack of OP-Cu on the ribose moiety of nucleotides followed by strand scission.[1–4,9,10] The character of the reactive species has not been finally established.[4] However, there is evidence that OP-Cu may generate diffusible hydroxyl radicals that attack riboses of nucleic acid by H-abstraction.[4,11–14] The structure specificity in OP-Cu-mediated cleavage of RNA originates from specific interactions of

[1] D. S. Sigman and C. B. Chen, *Annu. Rev. Biochem.* **59,** 207 (1990).
[2] C. S. Chow and J. K. Barton, *J. Am. Chem. Soc.* **112,** 2839 (1990).
[3] D. M. Perrin, A. Mazumder, and D. S. Sigman, *Progr. Nucleic Acid Res. Mol. Biol.* **52,** 23 (1996).
[4] W. K. Pogozelski and T. D. Tullius, *Chem. Rev.* **98,** 1089 (1998).
[5] G. J. Murakawa, C. B. Chen, W. D. Kuwabara, D. P. Nierlich, and D. S. Sigman, *Nucleic Acids Res.* **17,** 5361 (1989).
[6] Y.-H. Wang, S. R. Sczekan, and E. C. Theil, *Nucleic Acids. Res.* **18,** 4463 (1990).
[7] A. Mazumder, C. B. Chen, R. Gaynor, and D. S. Sigman, *Biochem. Biophys. Res. Commun.* **187,** 1503 (1992).
[8] T. Hermann and H. Heumann, *RNA* **1,** 1009 (1995).
[9] T. E. Goyne and D. S. Sigman, *J. Am. Chem. Soc.* **109,** 2846 (1987).
[10] O. Zelenko, J. Gallagher, Y. Xu, and D. S. Sigman, *Inorg. Chem.* **37,** 2198 (1998).
[11] L. M. Pope, K. A. Reich, D. R. Graham, and D. S. Sigman, *J. Biol. Chem.* **257,** 12121 (1982).
[12] H. R. Drew and A. A. Travers, *Cell* **37,** 491 (1984).
[13] T. B. Thederahn, M. D. Kuwabara, T. A. Larsen, and D. S. Sigman, *J. Am. Chem. Soc.* **111,** 4941 (1989).
[14] M. Dizdaroglu, O. I. Aruoma, and B. Haliwell, *Biochemistry* **29,** 8447 (1990).

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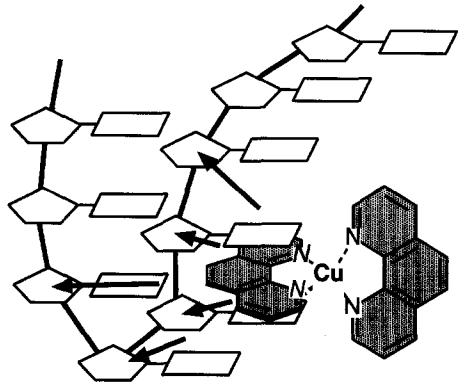


FIG. 1. The tetrahedral metal complex OP-Cu binds to nucleic acid and cleaves nucleotides surrounding the binding site (arrows). For RNA, the cleavage specificity of OP-Cu for single-stranded stacked regions was attributed to a "bookmarking" binding mode in which a single phenanthroline ligand of the metal complex partially intercalates into a stack of bases.

the metal complex with the nucleic acid.[8,15] For double-stranded helical conformations, the differences in reactivity found between A form RNA and B form DNA suggest that OP-Cu binds in the minor groove of the helix. While double-stranded DNA in B form is cleaved with high efficiency, A form nucleic acid, RNA as well as DNA, is inert to OP-Cu, probably due to the shallow shape of the minor groove in the A conformation, which prevents OP-Cu binding.[7,16]

Single-stranded RNA often forms highly ordered structures with extensive stacking interactions between neighboring bases. OP-Cu cleaves preferably within stacks of single-stranded nucleotides, as revealed in cases where the three-dimensional structure of the RNA is known from X-ray crystallography or nuclear magnetic resonance spectroscopy.[6,8] It has been proposed that OP-Cu binds to nucleotide stacks in RNA, like a bookmark, by partial intercalation of a single phenanthroline ligand between two adjacent bases (Fig. 1).[8] This binding mode is characterized by two strong cuts flanked by bands of decreasing intensity observed frequently in RNA cleavage patterns. The pattern is most likely generated by diffusible hydroxyl radicals, which radiate from the bound OP-Cu and cleave the RNA backbone. Because the radicals are "diluted" with increasing distance from the radical source, the cleavage intensity contains distance information. The observed signal intensity at a cleaved nucleotide roughly displays an inverse proportionality to the distance (1/r) between the radical source and the attacked

[15] D. S. Sigman, A. Spassky, S. Rimsky, and H. Buc, *Biopolymers* **24,** 183 (1985).
[16] G. J. Murakawa and D. P. Nierlich, *Biochemistry* **28,** 8067 (1989).

ribose. In the model system of the $tRNA^{Phe}$ anticodon loop, it has been shown that the $1/r$ dependence of the cleavage efficiency on the distance r of the cleaved nucleotide to the OP-Cu-binding site holds quantitatively.[8]

Based on the binding specificity of OP-Cu for stacked single-stranded nucleotides, OP-Cu probing provides information on the location of such regions in RNA. In addition, the $1/r$ dependence of the OP-Cu cleaving pattern can be used to determine relative distances between cleaved nucleotides. However, this is only feasible in cases where the cleavage pattern is generated from a single OP-Cu-binding site. Multiple overlapping OP-Cu-binding sites give rise to cleavage patterns that cannot be deconvoluted.

OP-Cu has been used successfully for secondary structure probing of RNA,[5–8] footprinting investigations on RNA/protein complexes,[16–19] and sequence-specific cleavage by oligonucleotide-linked OP-Cu.[19–21] This article describes the use of OP-Cu as a probe for RNA secondary structure and, if applicable, for determining relative distances between nucleotides within RNA folds.

Materials

Enzymes and Reagents

T4 RNA ligase and RNase T1 are from Roche, Penzberg, [5′-^{32}P]pCp is from Amersham Pharmacia, Freiburg

tRNA carrier is commercially available yeast $tRNA^{Phe}$ from Boehringer Mannheim

XC/BPB dye mix: 0.02% (w/v) xylene cyanol, 0.02% (w/v) bromphenol blue, 50% (v/v) glycerol

Buffers

3′-end-labeling buffer: 50 m*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) (pH 7.5), 10 m*M* $MgCl_2$, 10% (v/v) dimethyl sulfoxide (DMSO), 3 m*M* dithioerythritol (DTE)

1× TBE: 90 m*M* Trizma base, 90 m*M* boric acid, 1 m*M* EDTA; pH 8.3

[17] P. Darsillo and P. W. Huber, *J. Biol. Chem.* **266,** 21075 (1991).

[18] L. Pearson, C. B. Chen, R. P. Gaynor, and D. S. Sigman, *Nucleic Acids Res.* **22,** 2255 (1994).

[19] D. J. Bucklin, M. A. van Waes, J. M. Bullard, and W. E. Hill, *Biochemistry* **36,** 7951 (1997).

[20] J. Sun, J.-C. François, R. Lavery, T. Saison-Behmoaras, T. Montenay-Garestier, N. T. Thuong, and C. Hélène, *Biochemistry* **27,** 6039 (1988).

[21] C. B. Chen and D. S. Sigman, *J. Am. Chem. Soc.* **110,** 6570 (1988).

MG extraction buffer: 500 m*M* ammonium acetate, 10 m*M* magnesium acetate, 0.1 m*M* EDTA, 0.1% (w/v) sodium dodecyl sulfate (SDS)
Hydroxide hydrolysis buffer: 50 m*M* $NaHCO_3/Na_2CO_3$, 1 m*M* EDTA; pH 9.2
RNase T1 digestion buffer: 20 m*M* sodium acetate, 7 *M* urea, 1 m*M* EDTA; pH 5.0
OP-Cu probing buffer: 50 m*M* Tris–HCl, 10 m*M* $MgCl_2$, 50 m*M* NaCl; pH 8.0

Methods

Principle of Method

The 3′-end-labeled RNA is treated with the OP-Cu reagent under "single hit" conditions where each RNA molecule in the sample receives statistically less than one cut. The resulting RNA fragments are fractionated electrophoretically by size on polyacrylamide gels. The length of the separated fragments corresponds to the distance of the strand scission from the terminal radioactive label. Comparison of the fragment sizes with RNA "ladders," obtained by partial digestion with G-specific RNase T1 and by partial alkaline hydrolysis, allows to identify the positions of the cleaved nucleotides in the RNA molecule. Thus, stacked single-stranded regions in the RNA can be located directly by OP-Cu probing.

In cases where a cleavage signal can be attributed to a single OP-Cu-binding site, further quantification of band intensities may be used to determine relative distances between the cleaved nucleotides. Band intensities of the cleaved fragments are obtained by the integration of photometric scans of the X-ray autoradiograph or determined directly from PhosphoImager scans. Relative internucleotide distances r are calculated from the band intensities I using a simple inverse relation with a scalable proportionality factor q: $I = q/r$.

Probing Experiment

1. 3′-End-Labeling of RNA. Purified RNA is 3′-end-labeled with [^{32}P]pCp according to England *et al.*[22] The RNA precipitate is resuspended in water at a concentration of 1 mg/ml and kept on wet ice. For the labeling reaction, 1 μg of RNA (1 μl) is added to 30 μl labeling buffer containing 100 μM adenosine triphosphate (ATP), 10 μg/ml bovine serum albumin (BSA), 5 U of T4 RNA ligase, and 100 μCi of [5′-^{32}P]pCp (10 mCi/

[22] T. E. England, A. G. Bruce, and O. C. Uhlenbeck, *Methods Enzymol.* **65,** 65 (1980).

ml ~ 3000 Ci/mmol). After incubation at 15° for 6 hr (time may need to be adapated for different RNAs), RNA is precipitated by adding 300 m*M* sodium acetate (pH 5.4), 5 μg carrier tRNA, followed by 3 volumes of ice-cold ethanol. To complete precipitation, the mixture is cooled on dry ice for 5 min and centrifuged at 12,000–15,000*g* for 5 min.

2. Purification of Labeled RNA. The pellet of ^{32}P-labeled RNA from ethanol precipitation is rinsed with 70% (v/v) ethanol, air dried, and resuspended by vortexing in 5 μl water. After adding 5 μl of xylene cyanol (XC)/bromphenol blue (BPB) dye mix solution, the sample is loaded onto a polyacrylamide–8 *M* urea gel[23] (40 × 20 cm; thickness, 0.5 mm). Depending on the size of the RNA, gels of 10 or 20% (w/v) polyacrylamide [19% (w/v) acrylamide and 1% (w/v) *N,N'*-methylenebisacrylamide] in 1× TBE buffer are used. Electrophoresis is carried out at 500–600 V in 1× TBE buffer until the XC dye has moved to ~10 cm from the bottom of the gel (5–8 hr). An estimation can be made, considering that BPB and XC dyes migrate on 10% gels with RNAs of ~10 and ~60 nucleotides (nt), on 20% gels with RNAs of ~5 and ~25 nt, respectively.[24]

The band containing the labeled RNA is identified by autoradiography, cut out from the gel, and transferred to a microfuge tube. The gel slice is cut into small pieces and shaken at room temperature for at least 10 hr suspended in 300 μl of MG extraction buffer. The supernatant is transferred to a fresh tube and 10 μg of carrier tRNA is added, followed by 800 μl of ice-cold ethanol. After vortexing, the tube is cooled on dry ice for 5 min and centrifuged at 12,000–15,000*g* for 5 min. The pellet is washed at 4° with 70% (v/v) ethanol and resuspended in 50 m*M* Tris–HCl buffer (pH 7.5). Aliquots are removed, corresponding to 50,000 cpm for the following OP-Cu probing reaction (see later), 20,000 cpm for the alkaline hydrolysis sequence ladder, and 10,000 cpm for the RNase T1 digestion. The remaining RNA is ethanol precipitated and stored at −20°.

3. Partial Alkaline Hydrolysis of RNA. An aliquot of ~20,000 cpm ^{32}P-labeled RNA is mixed with 10 μg carrier tRNA and 10 μl of hydroxide hydrolysis buffer. The sample is incubated at 90° for 5 min and then immediately put on ice for further use in producing a sequence ladder in polyacrylamide gel electrophoresis of the RNA fragments from OP-Cu cleavage.

4. G-Specific RNase T1 Partial Digestion of RNA. To an aliquot of ~10,000 cpm ^{32}P-labeled RNA, 2 μg carrier tRNA, 10 μl digestion buffer, and 0.02 U of RNase T1 are added. The reaction mixture is incubated at 55° for 10 min and then kept on ice for later use in producing a G ladder in gel electrophoresis.

[23] A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65,** 499 (1980).
[24] A. Krol and P. Carbon, *Methods Enzymol.* **180,** 212 (1989).

5. OP-Cu Probing Reaction. A solution of ~50,000 cpm ^{32}P-labeled RNA is mixed with 100 ng carrier tRNA and 20 μl of probing buffer. After addition of the OP-Cu reagent solution, the final reaction volume should be ~50 μl. The OP-Cu reagent is freshly prepared from 1,10-phenanthroline and copper(II) sulfate (2:1) to a final concentration of 40 and 20 μ*M*, respectively (concerning the concentration of OP-Cu, see the remark on the evaluation of probing data for distance determination). To the reaction mixture, hydrogen peroxide is added to a final concentration of 7 m*M*. The reaction is started by the addition of 5 m*M* of the reducing agent 3-mercaptopropionic acid (MPA). In order to achieve rapid mixing of the reactants, it is convenient to carefully set droplets of H_2O_2 and MPA at the wall of the microfuge tube containing the mixture of RNA and OP-Cu reagent. The reaction is then started by centrifuging briefly to spin down the droplets. The probing reaction is allowed to proceed for 2–5 min at 37°. The optimal reaction time must be adapted for each RNA target, depending on the achieved cleavage rate. The probing reaction is quenched by adding an excess of 2,9-dimethyl-1,10-phenanthroline (neocuproine) solution to a final concentration of 1 m*M*. Neocuproine is a strong chelating agent that forms oxidatively stable complexes with Cu(II).[25]

The cleaved RNA is precipitated by adding 3 volumes of ethanol in the presence of 300 m*M* sodium acetate (pH 5.4). After centrifuging and reprecipitating from 100 μl of 300 m*M* sodium acetate, the pellet is rinsed with 100 μl of ice-cold 70% (v/v) ethanol and air dried.

6. Analysis of Cleaved RNA. For the electrophoretic fractionation of cleavage fragments, the RNA pellet is dissolved in 5 μl of water and 5 μl of XC/BPB dye mix is added. The sample is loaded onto a polyacrylamide–8 *M* urea sequencing gel (length, 40 cm; thickness, 0.5 mm) made in 1× TBE buffer. Depending on the length of the intact RNA, gels of 10–15% (w/v) polyacrylamide [19% (w/v) acrylamide and 1% (w/v) *N,N'*-methylenebisacrylamide] are used. Gels of higher percentage (BPB dye runs halfway from the start) are suitable to resolve the short RNA fragments resulting from cleavage close to the 3′ end label. Gels are prerun at 40 W in order to improve band resolution by both warming the gel and equilibrating ionic differences between the gel and the buffer reservoirs. Prerunning is done for 1–2 hr until the BPB in a preloaded dye sample has moved halfway down from the start. Electrophoresis of the RNA fragments is carried out at 40–70 W for times adapted to the size of the RNA to be analyzed. For an estimation, it can be considered that XC dye migrates to ~60 nt on 10% gels.[24]

[25] D. R. Graham, L. E. Marshall, K. A. Reich, and D. S. Sigman, *J. Am. Chem. Soc.* **102,** 5419 (1980).

In order to obtain reference sequence ladders, reaction mixtures of partial alkaline hydrolysis and partial RNase T1 digestion are electrophoresed together with the cleavage fragments. For the reference lanes, 4 μl of each reaction mixture is loaded onto the gel. Sequence reading is facilitated by applying two lanes of partial alkaline hydrolysis flanking the OP-Cu probing reactions and partial T1 digestion. It is recommended to run at least one control lane where RNA is loaded, which was kept in probing buffer without the added OP-Cu reagent. Workup of the control sample by RNA precipitation is identical to the OP-Cu-treated reaction mixture. If the OP-Cu cleavage pattern is quantified for distance determination (see later), the control lane is used for background subtraction and should thus contain the same amount of ^{32}P-labeled RNA as is loaded for the probing experiment.

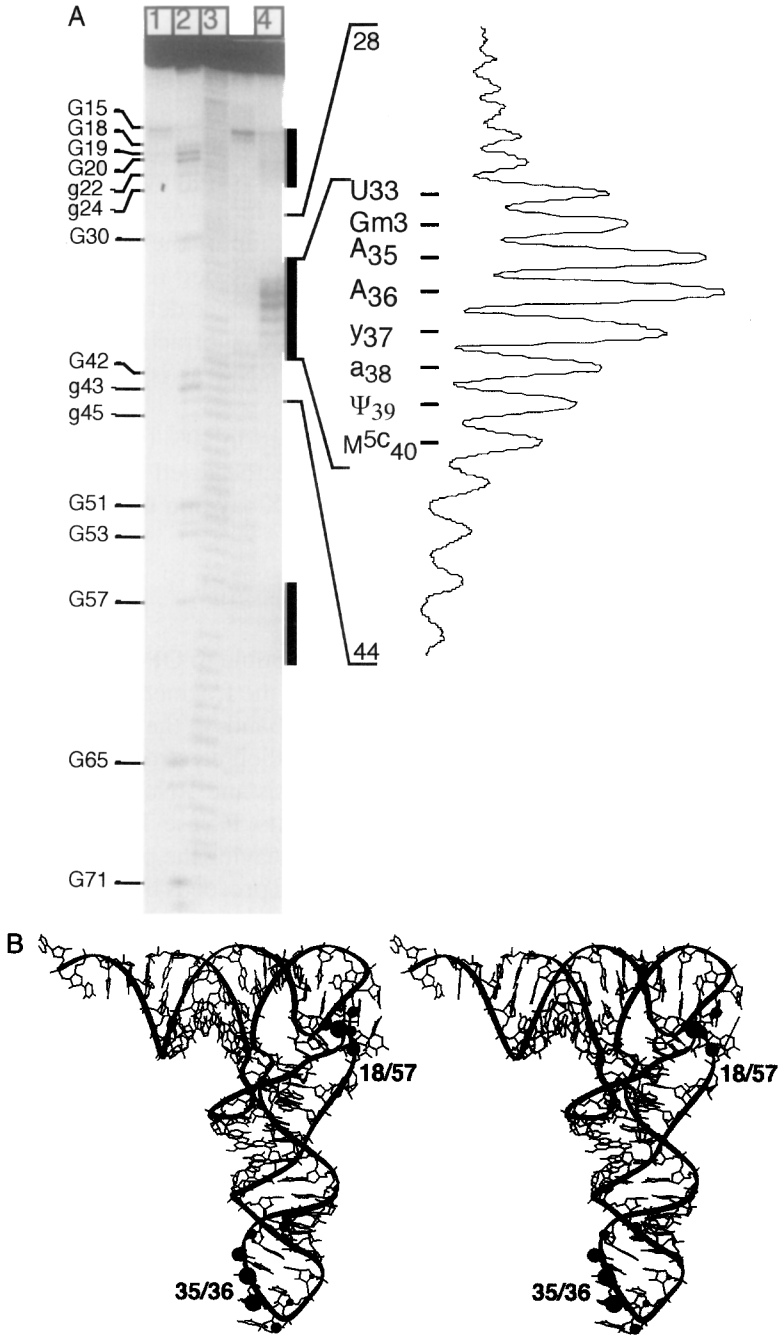
At the end of electrophoresis, the gel is fixed in 10% (v/v) acetic acid and dried *in vacuo*. An autoradiograph of the the dried gel is recorded either by PhosphoImaging or by exposition to X-ray film using an intensifying screen.

Evaluation of Probing Data

1. Structural Information. Nucleotides susceptible to OP-Cu scission are identified on the autoradiograph by comparing the fragments from OP-Cu probing with sequence ladders. When assigning bands of the different cleavage reactions, the nature of the fragments, thus their electrophoretic mobility, must be considered. Both alkaline hydrolysis and RNase T1 digestion produce 5′-OH ends at 3′-end-labeled fragments. RNase T1 cleaves GpN phosphodiester bonds 3′ to unpaired guanines, leaving the phosphate at the unlabeled 5′ fragment. Strand scission by OP-Cu proceeds by elimination of the susceptible nucleoside, leaving phosphate groups at both the 3′ and the 5′ fragment.[9] Therefore, both cleavage reactions result in products that differ slightly in their electrophoretic mobility.

Cleavage of RNA by OP-Cu typically yields clusters of fragments that display decaying intensity centered around one or two strong bands (Fig. 2A). The example of tRNA^{Phe}, for which a high-resolution crystal structure is available,[26] shows that unpaired nucleotides are cleaved preferentially in regions where base stacking occurs.[5,8] Molecular modeling studies have suggested that OP-Cu binds to stacked single-stranded regions within RNA folds by partial intercalation of a single phenanthroline ligand between two adjacent bases (Fig. 1).[8] Two major cleavage sites are observed in tRNA^{Phe}, namely in the D/T loop junction around G18/G57 and in the anticodon

[26] E. Westhof, P. Dumas, and D. Moras, *Acta Crystallogr. A* **44,** 112 (1988).


A
1
2
3
4
G15
G18
G19
G20
g22
g24
G30
G42
g43
g45
G51
G53
G57
G65
G71
28
U33
Gm3
A35
A36
y37
a38
Ψ39
M5c40
44
B
18/57
35/36
18/57
35/36

loop around A35/A36 (Fig. 2).[5,8] The three-dimensional structure reveals extensive stacking interactions of the bases in both loop regions (Fig. 2B).[26] In contrast, low reactivity toward OP-Cu cleavage is found in the single-stranded variable loop where less stacking occurs.

2. *Distance Information.* Relative internucleotide distances in RNA folds may be determined using the OP-Cu cleavage pattern, which are likely to originate from single nonoverlapping OP-Cu-binding sites. Such sites are recognized by a well-defined intensity maximum of one to three bands flanked on both sides by nucleotides cleaved with gradually decreasing efficiency.[8] Multiple and unspecific binding of OP-Cu to RNA is reduced by applying low concentrations of the metal complex in the probing experiment. The concentration of OP-Cu given earlier, being 10-fold lower than concentrations occasionally reported in the literature, yielded an optimal single-site probing pattern for $tRNA^{Phe}$.[8] However, the OP-Cu concentration applied in the probing experiment is a parameter that must be optimized for each RNA target, especially if probing data are to be quantified. If OP-Cu is used exclusively for nonquantitative probing of the RNA secondary structure, up to 10-fold higher concentrations of the reagent may be applied.

Cleavage pattern are quantified either directly from PhosphoImager data or, if X-ray film is used, by photodensitometric scanning of autoradiographs. For photodensitometry, lightly exposed films are desirable on which the bands of interest are within the nonsaturating density range of the X-ray film (Fig. 2A). The lane(s) of the OP-Cu probing reaction and a control lane with uncleaved RNA are scanned. Densities of OP-Cu-cleaved fragment bands are corrected for background by subtracting the scanned densities in the same region of the control lane. Numerical integration of the peak areas under the density scan is performed to calculate the probability of cleavage at the reactive nucleotides. Peak area integration can be done directly in the analysis software of the PhosphoImager or photodensitome-

FIG. 2. (A) Autoradiograph of the cleavage fragments obtained by OP-Cu probing of 3′-end-labeled $tRNA^{Phe}$. Lane 1, control with untreated $tRNA^{Phe}$; lane 2, G-specific RNase T1 digestion; lane 3, alkaline hydrolysis; lane 4, OP-Cu cleavage; unlabeled lane, control with Fe-EDTA-generated OH-radicals. The regions of enhanced cleavage around G18/G57 and A35/A36 are marked with bars. A low exposition time was chosen here in order to yield a nonsaturating signal suitable for quantification for the A35/A36 cleavage site. As a consequence, the G18/G57 signals are weak on this autoradiograph. On the right, a densitometric scan of the OP-Cu cleavage reaction (lane 5) is shown for the nucleotides C28–A44. (Adapted from Hermann and Heumann.[8]) (B) Stereo view of the three-dimensional structure of $tRNA^{Phe}$ with spheres marking nucleotides cleaved by OP-Cu. Sphere size indicates cleavage probability at the two major cleavage sites designated 18/57 and 35/36.

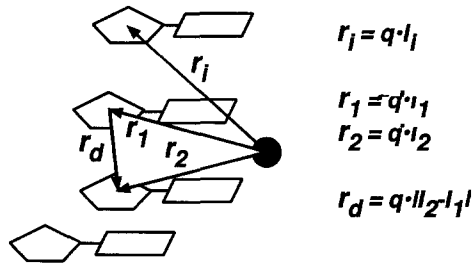


FIG. 3. Principle of distance determination using OP-Cu cleavage pattern (see also Fig. 1). The proportionality of the cleavage probability I at a nucleotide i and the distance r_i between the nucleotide and the OP-Cu reagent (black circle) allows the calculation of relative distances between nucleotides even without knowing the proportionality factor q.

ter or with the help of common data analysis programs (CricketGraph, DeltaGraph, Igor, KaleidaGraph, etc.).

Distances r_i between the bound OP-Cu and the cleaved nucleotides i are obtained from peak areas I of the corresponding RNA fragment bands according to the proportionality $r_i = qI_i$. Because the proportionality factor q is not known *a priori,* relative internucleotide distances can be obtained by forming differences between pairs of nucleotides (Fig. 3). For a rough estimation of absolute distances, it can be assumed that if OP-Cu binds by partial intercalation, the Cu(II) atom of OP-Cu is located approximately 5–6 Å away from the center of the nearest nucleotide sugars immediately flanking the OP-Cu-binding site. Thus, q may be calculated from the cleavage probability determined by the intensity of the strongest cut at an assumed OP-Cu-binding site. Other absolute nucleotide distances are then calculated using this q value.

Relative internucleotide distances obtained from OP-Cu probing data will at first yield information whether the probed RNA region deviates from a straight conformation.[8] If nucleotides are bending away from an OP-Cu-binding site, their distances to the reactive Cu(II) center and thus the probing signal will change more markedly than in a straight assembly. Second, internucleotide distances may be used in modeling of the RNA three-dimensional structure. RNA structure modeling calls for a combination of data from different theoretical and experimental approaches, such as comparative phylogeny, chemical and enzymatic probing, cross-linking, mutational analysis, and ligand-binding studies.[27–29] While the few distance

[27] E. Westhof and F. Michel, *in* "RNA-Protein Interactions" (K. Nagai and I. W. Mattaj, eds.), pp. 25–51. IRL Oxford Univ. Press, Oxford, 1994.
[28] P. Romby, this volume.
[29] T. Hermann and E. Westhof, *Curr. Opin. Biotech.* **9,** 66 (1998).

constraints obtained from OP-Cu probing are not sufficient to fully determine the three-dimensional structure of an RNA target, they provide useful information on the local folding of the nucleic acid. This knowledge is especially valuable considering that the RNA three-dimensional structure is assembled from modular units.[30,31]

Acknowledgments

Matthias Götte is thanked for providing probing data. T.H. was supported by an EMBO long-term fellowship. H.H. thanks the Deutsche Forschungsgemeinschaft for support (He 1285/8-3).

[30] F. Michel and E. Westhof, *Science* **273,** 1676 (1996).
[31] E. Westhof, B. Masquida, and L. Jaeger, *Fold. Des.* **1,** 78 (1996).

[4] Applications of Uranyl Cleavage Mapping of RNA Structure

By NIELS ERIK MØLLEGAARD and PETER E. NIELSEN

Introduction

The uranyl(VI) ion, UO_2^{2+}, binds very strongly to DNA and, on irradiation with long wavelength UV light, induces single-strand breaks.[1,2] The excited state of UO_2^{2+} is a very strong oxidant, and DNA cleavage patterns support a mechanism in which a uranyl ion coordinated to a phosphate group oxidizes proximal sugars via a direct electron-transfer mechanism.[2]

Thus uranyl cleavage of DNA reflects phosphate accessibility as well as affinity for the uranyl ion. Therefore, uranyl photoprobing can be employed to study protein(ligand)–DNA interactions in terms of phosphate–protein contacts[1,3–5] as well as conformational variations in the DNA double

[1] C. Jeppesen, O. Buchardt, and P. E. Nielsen. *FEBS Lett.* **235,** 122 (1988).
[2] P. E. Nielsen, C. Hiort, O. Buchardt, O. Dahl, S. H. Sönnichsen and B. Nordén, *J. Amer. Chem. Soc.* **114,** 4967 (1992).
[3] C. Jeppesen and P. E. Nielsen, *Nucleic Acids Res.* **17,** 4947 (1989).
[4] N. E. Møllegaard, P. E. Rasmussen, P. Valentin-Hansen, and P. E. Nielsen, *J. Biol. Chem.* **268,** 17471 (1993).
[5] N. E. Møllegaard and P. E. Nielsen, *Methods Mol. Biol.* **90,** 43 (1997).

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