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Use of Low-Molecular-Weight Polyethylene Glycol in the Crystallization of RNA Oligomers

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Abstract

We have crystallized a variety of RNA oligonucleotides in a form suitable for X-ray diffraction studies using polyethylene glycol with a low-molecular-weight distribution (PEG 400) as the precipitant. Crystallization experiments on a set of 26 RNA oligomers ranging from eight to 12 nucleotides in length resulted in eight diffraction-quality crystals. Of these eight RNA crystals, six utilized PEG 400 as the precipitating agent. We have also been able to obtain large single crystals of a DNA–RNA hybrid, transfer RNA (two different conditions) and a catalytic RNA from PEG 400 solutions. These results suggest that PEG 400 may be a generally useful alternative to 2-methyl-2,4-pentanediol (MPD) which has, thus far, been the most successful precipitant for DNA oligomers.

Introduction

The time is now ripe for a focused effort toward increasing our understanding of the structure of RNA in biological systems. Recent studies have shown that RNA is capable of many roles previously assigned only to proteins. These include catalytic activity, structural scaffolding and signal transmission by molecular recognition. Just as with proteins, a complex tertiary structure is necessary to enable RNA to perform these functions. Since the analysis of the tRNA structure by X-ray crystallography in the late 1970s, progress in structural determination of RNA has been exceedingly slow largely due to difficulties in synthesizing milligram quantities of homogeneous RNA and obtaining crystals of suitable size and quality for X-ray diffraction experiments. Recent developments in RNA synthesis by transcription from DNA templates and by chemical synthesis from monomers have to a great extent solved the problem of supply of the large quantities of RNA oligomers necessary for crystallographic (and NMR) structural studies (Usman, Egli & Rich, 1992; Wyatt, Chastain & Puglisi, 1991).

Methods and conditions used for the preparation of diffraction-quality crystals of nucleic acids have been reviewed (Holbrook & Kim, 1985; Jancarik & Kim, 1991). By far the most successful precipitant to date has been 2-methyl-2,4-pentanediol (MPD). MPD has been used both for crystallization of transfer RNAs and for many DNA oligomers and an RNA oligomer (Dock-Bregeon *et al.*, 1989). Other precipitants such as isopropanol, acetone, dioxane and polyethylene glycol have been used for the crystallization of some nucleic acids. Polyethylene glycol of average molecular weight 400 has been used for the crystallization of some drug–DNA complexes, but has not been generally reported to be a useful reagent in the crystallization of RNA molecules (Wang & Gao, 1990). Based on crystallization trials of over 30 RNA and DNA molecules, we report here our empirical observation of the utility of a low-molecular-weight polyethylene glycol (PEG 400) as a precipitant in the crystallization of RNA oligonucleotides.

Materials and methods

As part of our ongoing structural studies of RNA oligomers by X-ray crystallography, we are developing effective sets of screening conditions for the growth of single RNA crystals. We have tested two sets of crystallization screens: (*a*) the relatively high salt conditions of Jancarik & Kim (1991) and (*b*) a new set of conditions, referred to as the NACRYST screen, many of which were previously observed to produce crystals of DNA oligomers and transfer RNA and which

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Table 1. *Nucleic acid crystallization screen – NACRYST*

	Monovalent cation	Divalent cation	Multivalent cation	Buffer	Precipitant
1.	Na	Mg	Spermine	Cacodylate	35% MPD
2.	—	Mg	—	HEPES	35% MPD
3.	K	—	Spermine	Tris	30% MPD
4.	K	Mn	—	HEPES	35% MPD
5.	NH_4	Ba	—	Cacodylate	30% MPD
6.	Na	Cd	—	Cacodylate	35% MPD
7.	Na	Zn	—	HEPES	30% MPD
8.	—	—	—	Cacodylate	15% Isopropanol
9.	Na	Mg	—	—	15% Isopropanol
10.	—	Cd	Spermine	Tris	15% Isopropanol
11.	NH_4	—	—	HEPES	30% PEG 400
12.	Na	Mg	Spermine	Cacodylate	35% PEG 400
13.	Na	Mn	—	Tris	35% PEG 400
14.	K	Cd	—	Cacodylate	35% PEG 400
15.	NH_4	Mg	—	HEPES	35% PEG 600
16.	Na	Mg	—	Cacodylate	30% PEG 1000
17.	-	Mg	Spermine	Cacodylate	35% PEG 4000
18.	Na	—	—	Tris	35% PEG 8000
19.	K	Mg	—	HEPES	1.2 *M* Ammonium sulfate
20.	Na	—	—	Cacodylate	1.2 *M* Ammonium sulfate
21.	K	Mg	—	Cacodylate	1.2 *M* Sodium acetate
22.	-	Ba	—	HEPES	1.2 *M* Sodium acetate
23.	Na	—	—	HEPES	40% *t*-Butanol
24.	–	Mg	Spermine	—	15% Acetone
25.	Li	Mg	—	Cacodylate	35% MPD
26.	Arg.	Mn	Sm^{3+}	Cacodylate*	30% MPD
27.	Na	Zn	—	Tris*	30% MPD
28.	—	—	Ru^{3+}	Tris	30% MPD
29.	Arg.	Co(hex)	—	HEPES	30% MPD/10% PEG 1000
30.	NH_4	—	Putrescine	Cacodylate*	30% MPD/10% PEG 400
31.	K	Mg	—	Tris*	30% MPD/5% isopropanol
32.	—	Ba	—	Cacodylate*	20% Ethanol
33.	NH_4	—	Sm^{3+}	Tris*	20% Ethanol
34.	Arg.	Ba	Spermidine	Cacodylate*	30% PEG 400
35.	K	Ca	—	Cacodylate	30% PEG 400
36.	—	Co(hex)	—	HEPES	35% PEG 400
37.	Na	—	Ru^{3+}	Tris*	35% PEG 400
38.	Li	Co	—	Cacodylate	35% PEG 600
39.	—	Co(hex)	Putrescine	—	35% PEG 600
40.	Arg.	Cd	—	Cacodylate*	30% PEG 1000
41.	—	Ba	Sm^{3+}	Cacodylate*	35% PEG 4000
42.	Na	—	Ru^{3+}	Tris*	35% PEG 8000
43.	—	Zn	—	HEPES	35% PEG 8000
44.	—	Ca	Spermidine	Cacodylate	20% Dioxane
45.	Li	Co	—	Cacodylate*	20% Dioxane
46.	NH_4	—	—	Tris	20% Dioxane
47.	—	Cd	Spermine	HEPES	40% *t*-Butanol
48.	K	Ba	—	Tris	15% Acetone

Salt (all salts contain the chloride anion)	Concentration (m*M*)
Monovalent ions	20
Divalent ions (Mg, Mn, Zn, Ca, Co)	5
Other divalent ions (Ba, Cd)	1
Polyamines (spermine, spermidine, putrescine)	2
Cobalt hexammine $[Co(hex)]^{2+}$ Cl, Ru^{3+}, Sm^{3+}	1
Argininamide (Arg.)	5

Buffer concentration: HEPES, Tris, cacodylate, 50 m*M*
Buffer pH: Cacodylate* 6.0, cacodylate 6.5, HEPES 7.0, Tris 7.5, Tris* 8.0
Precipitants: MPD (2-methyl-2,4-pentanediol); PEG (polyethylene glycol)

are characterized by low overall salt concentration. The NACRYST crystallization screen is detailed in Table 1. For each of the 50 conditions of the Jancarik matrix and the 48 conditions of the NACRYST matrix, a different combination of monovalent cation, divalent cation, polyvalent cation, buffer and precipitant was used. In some of the conditions a monovalent, divalent or polyvalent cation or even a buffer is omitted entirely, while in others more than one of the same type of ion or precipitant is used. The wide range of chemicals used in the crystallization trials emphasizes the significance of the similarity of conditions actually found to produce crystals.

A total of eight of the 48 (17%) conditions in the NACRYST screen and four of the 50 in the Jancarik matrix (8%) use PEG 400 as a precipitant. PEG 400 from

Table 2. *RNA molecules crystallized from PEG* 400

RNA sequence 5′→3′	Buffer* pH	Added salt concentration	Precipitant concentration†	Crystal shape and size (mm) Space group	Diffraction resolution (Å)
GGACUUCGGUCC	50 m*M* Tris pH 8.5	100 m*M* Na Citrate 2.5 m*M* $MgCl_2$	PEG 400 12→30%	Plates 1 × 1 × 0.1 *C*2	2.0
CGCAGGCG	100 m*M* HEPES pH 7.5	200 m*M* $ClCl_2$	PEG 400 10→20%	Bipyramidal 0.3 × 0.1 × 0.1 $P6_122$ or enantiomorph	3.0
CGCAIGCG	100 m*M* HEPES pH 7.5	200 m*M* $CaCl_2$	PEG 400 10→20%	Bipyramidal 0.3 × 0.1 × 0.1 $P6_122$ or enantiomorph	3.0
GGCCGAAAGGCC	50 m*M* Tris pH 7.5	20 m*M* NaCl 5 m*M* $MnCl_2$	PEG 400 15→30%	Hexagonal 0.3 × 0.2 × 0.2 $P6_122$ or enantiomorph	2.3
GGCGAGCC	100 m*M* HEPES pH 7.5	5 m*M* $MgCl_2$	1→2 *M* $(NH_4)_2SO_4$ 2→4% PEG 400	Hexagonal 0.8 × 0.2 × 0.2 $P6_2$ or enantiomorph	3.0
GICGAGCC	100 m*M* HEPES pH 7.5	5 m*M* $MgCl_2$	1→2 *M* $(NH_4)_2SO_4$ 2→4% PEG 400	Hexagonal 0.8 × 0.2 × 0.2 $P6_2$ or enantiomorph	2.7
Hammerhead catalytic RNA‡	35 m*M* NaCac pH 6.5	28 m*M* $MgCl_2$ 19 m*M* Spermine	PEG 400 6→18%	Plates 0.5 × 0.2 × 0.2 *P*1	2.4
d-CTTTTG: r-CAAAAG	100 m*M* HEPES pH 7.4	200 m*M* $MgCl_2$	PEG 400 15→30%	Plates 0.8 × 0.7 × 0.1 —	Not measured
Yeast $tRNA^{Phe}$ (condition 1)	100 m*M* HEPES pH 7.4	200 m*M* $CaCl_2$	PEG 400 14→28%	Plates 0.5 × 0.5 × 0.2 —	Not measured
Yeast $tRNA^{Phe}$ (condition 2)	100 m*M* HEPES pH 7.4	200 m*M* $MgCl_2$	PEG 400 15→30%	Bipyramidal 1.0 × 0.3 × 0.1 —	Not measured

* NaCac, sodium cacodylate.
† Drop concentration→reservoir concentration.
‡ See Kim *et al.* (1994).

Fisher Scientific was used for all initial experiments both in the reservoir and the crystallization drop. In some cases chromatographic grade Ultrapure PEG 400 from EM Science (VWR) was used in the crystallization drop and resulted in improved crystals.

Initial crystallization experiments were performed by the hanging-drop vapor-diffusion technique using drop volumes of 2–4 μl and reservoir volumes of 0.7 ml. Some refinement of crystallization conditions was performed by the sitting-drop method and larger volumes were used. The RNA oligomers were dissolved in water (0.1 m*M* EDTA), normally to a concentration of 2 m*M* (1–4 m*M* concentrations were tried) and mixed with an equal volume of the reservoir solution against which the drop was then equilibrated. Most experiments were performed at room temperature, with a few exceptions which were also attempted in a 277 K cold room.

Results and discussion

Of 26 RNA and nine DNA oligomers tested with the Jancarik screen (Jancarik & Kim, 1991) and the NACRYST screen described in Table 1, eight RNAs and two DNAs were crystallized. Of the eight RNAs, six were crystallized using PEG 400 as a precipitant, one was crystallized from MPD and one from PEG 4000. The two DNAs were crystallized from PEG 8000. Table 2 summarizes the RNA crystals obtained from conditions having PEG 400 as precipitant (or co-precipitant). As shown in this Table, crystals of a DNA–RNA hybrid and two crystal forms of yeast $tRNA^{Phe}$ have also been obtained from PEG 400. These molecules have previously been crystallized under different conditions using MPD or isopropanol as the precipitant (Holbrook, Martin, Tinoco, Young & Kim, 1981; Kim *et al.*, 1973). Also, one of us (JJ) has recently been successful in crystallizing a 'hammerhead' self-cleaving RNA using PEG 400 as precipitant (Kim *et al.*, 1994).

The consensus of the successful crystallization conditions shown in Table 2 requires a buffer of pH 7.5 (one each at pH 6.5 and 8.5), a divalent cation (Mg^{2+}, Mn^{2+} and Ca^{2+}) as well as a PEG 400 gradient of 6–15% in the drop and 18–30% in the reservoir. The table also shows that a lower concentration of PEG 400 is needed when $(NH_4)_2SO_4$ is the co-precipitant.

The effectiveness of polyethylene glycol as a precipitant in the crystallization of proteins (Ducruix & Geigé, 1992) has been well established with about 40% of the compiled protein crystallizations utilizing this reagent. However, there have been few reports of the use of PEG in the crystallization of nucleic acids. Crystals of the Z-form DNA oligomer $d(CG)_3$ were obtained from PEG 600 (Wang, Hakoshima, van der Marel, van Boom & Rich, 1984) and Wang (Wang & Gao, 1990) has also used PEG 400 in the crystallization of some drug–DNA complexes in which the drugs may not be soluble in alcohols such as MPD. There has also been some success in the use of PEG 400 in obtaining crystals of protein–DNA complexes (Joachimiak & Sigler, 1991).

Interestingly, the most successful PEG in the crystallization of proteins has been of a medium size (M_r = 2000–8000), roughly one-sixth the size of an aver-

age protein. This study shows that in the case of the smaller RNA oligomers, polyethylene glycol of average molecular weight 400, roughly one-tenth the size of an RNA dodecamer single strand is most effective as a crystallization reagent. In both cases, a much smaller size PEG than the molecule of interest is most effective in inducing crystallization.

The success of PEG 400 in the crystallization of RNA may be related to its relatively low molecular weight, lack of charge, and miscibility in water. The apparent preference for PEG 400 in the crystallization of RNA *versus* MPD in the crystallization of DNA may be due to the presence of the O2′ hydroxyl groups along the surface of the molecule. Another possibility is simply that PEG 400 has not been widely used as a DNA crystallization reagent and, therefore, few crystals have been obtained. Hopefully, this report will stimulate further testing of this promising approach.

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