Acta Cryst. (1996). D52, 465–468

A Highly Efficient 24-Condition Matrix for the Crystallization of Nucleic Acid Fragments

IMRE BERGER,[a,b] CHULHEE KANG,[a,c] NANDA SINHA,[d] MARK WOLTERS[a] AND ALEXANDER RICH*[a]

[a]*Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02141, USA,* [b]*Department of Biophysical Chemistry, Hannover Medical School, 30623 Hannover, Germany,* [c]*Department of Biochemistry and Biophysics, Washington State University, Pullman, Washington 99164-4660, USA, and* [d]*Millipore Corporation, Bedford, MA 01730, USA*

(*Received 17 July 1995; accepted 3 October 1995*)

Abstract

A matrix screen is presented with optimized conditions for the crystallization of nucleic acid fragments. The screen consists of 24 conditions only and utilizes minimal amounts of sample. It has been tested on a series of DNA and RNA oligomers, and yielded diffraction-quality crystals of many specimens including drug–DNA complexes. Preliminary X-ray analysis of the crystals is reported.

1. Introduction

Since the development of an efficient chemical synthesis method for deoxy oligonucleotides more than two decades ago, a large number of DNA oligomers have beeen crystallized and their structures solved at high resolution (reviewed in Dickerson, 1991). X-ray diffraction analysis of single crystals led to a better understanding of the geometry of the B and A conformation of DNA predicted earlier based on fiber-diffraction studies (Dickerson *et al.*, 1982; Dickerson, Drew, Conner, Kopka & Pjura, 1983; Watson & Crick, 1953; Leslie, Arnott, Chandrasegaran & Ratliff, 1980) and at present the crystal structures of more than 200 RNA and DNA fragments have been determined (Joshua-Tor & Sussmann, 1993; Wahl & Sundaralingam, 1995). In addition, single-crystal diffraction analysis of $d(CG)_3$ led to the discovery of the left-handed Z-DNA conformation (Wang *et al.*, 1979). More recently, the crystal structure of $d(G_4T_4G_4)$ showed the geometry of four-stranded telomeric DNA (Kang, Zhang, Ratliff, Moyzis & Rich, 1992) and the high-resolution (1.2 Å) single-crystal analysis of $d(TG_4T)$ made possible the visualization of many details of parallel-stranded guanine-rich quadruplex DNA (Laughlan *et al.*, 1994). A recent example for DNA polymorphism is the occurrence of four-stranded intercalated cytosine-rich DNA, proposed in 1993 based on NMR data (Gehring, Leroy & Gueron, 1993). The single-crystal analysis of $d(C_4)$ (Chen, Cai, Zhang & Rich, 1994) and in particular the 1.4 Å crystal structure of $d(C_3T)$ (Kang *et al.*, 1994) revealed this novel motif at high resolution.

In spite of the considerable number of nucleic acid crystals obtained, the crystallization of oligonucleotides still remains an empirical often random process. Nevertheless, at least in the case of deoxy nucleic acids, a careful analysis of the reported crystallization conditions reveals a number of reoccurring ingredients which appear to enhance the probability of obtaining highly ordered single crystals suitable for diffraction analysis (Dickerson, 1991; Wang & Gao, 1990; Dock-Bregeon & Moras, 1991). Using these ingredients, we have developed a crystallization screening method for nucleic acid oligomers composed of only 24 conditions. We were able to crystallize a large number of DNA oligonucleotides from these conditions, using minimal amounts of material (less than 100 µl of 1–4 m*M* oligonucleotide stock solutions). In most cases, the single crystals diffract to high resolution and made possible the elucidation of the molecular structure. We have also applied the crystallization screen to RNA oligonucleotides, and we were able to obtain crystals from a number of RNA oligomers tested.

2. Materials and methods

DNA and RNA oligomers were synthesized on an Applied Biosystems DNA synthesizer. The sequences were heated to 358 K for 1 h and subsequently purified by high-pressure liquid chromatography (HPLC) on a preparative column at 343 K applying a linear gradient of 15–45% acetonitrile in 0.1 m*M* TEAA (triethylammoniumacetate) buffer over a period of 35 min. The oligomer solutions were passed through a 0.45 µm syringe filter unit (Uniflo®, Schleicher and Schuell, Keene, NH, USA), lyophilized and resuspended several times. Then they were stored in aliquots of 10 m*M* (single-strand concentrated) stock solutions at 277 K. For crystallization trials, the stock solutions were diluted depending on the length and solubility of the particular sequence. 24-well tissue-culture plates (Linbro®, Flow Laboratories Inc., McLean, VA, USA) were used applying the hanging-drop method, and two droplets were suspended over a reservoir of 500 µl 35% MPD (2-methyl-2,4-pentanediol). The droplets contained 1 µl + 2 µl and 2 µl + 2 µl of nucleic acid solution and reagent stock solution, respectively.

Initially, identical sets of trays were prepared for incubation at 277 K and at room temperature (290 K).

However, the setups incubated at 277 K proved to be more successful in yielding diffraction-quality crystals and thus the room-temperature option was discarded in an effort to minimize the amount of sample consumed. The plates were monitored over a course of several weeks. The results were tabulated according to the initial conditions.

3. Results and discussion

3.1. *Design of matrix*

The variable parameters were pH, concentration of monovalent cations, magnesium ions, other divalent cations, polyamines and cobalt hexammine. MPD was used as the only precipitating agent, usually at a concentration of 30 or 35%(*v/v*). Depending on the monovalent cation in the individual conditions, either potassium or sodium cacodylate buffer with the appropriate pH was chosen. The resulting matrix for the crystallization trials is given in Table 2. Buffers, salt and polyamine solutions (Table 1) were prepared in quantities of 50 ml, filtered through 0.2 µm bottle-top filters (Zapcap®-S, Schleicher and Schuell, Keene, NH, USA) and stored at 277 K. Boxes of 24 eppendorf tubes each with a volume of 1 ml containing the various stock solutions listed in Table 2 were used for crystallization screens and stored at 277 K in between the trials. The crystallization plates were set up at room temperature and subsequently stored at 277 K for incubation.

3.2. *Crystal growth*

We used a variety of DNA and RNA oligomers to screen for crystal growth. Prior to crystallization, the stock solutions of the oligomers were diluted to a concentration of roughly 20 to 24 m*M* '(mono-)nucleotide' concentration per ml (thus, in the case of a dodecamer, the single-strand concentration was 2 m*M*). No further incubation was carried out. If significant precipitation was observed in more than 30% of the droplets within a period of several minutes after adding the components, the initial concentration of the nucleic acid oligomer was reduced by half. Crystals were obtained at 277 K after periods of a few days to weeks. The crystals obtained were of diffraction quality and in general no further refinement of the crystallization conditions was necessary. In some cases, however, a 'feeding' technique was applied to improve the size of the crystals. Thus, the sealed wells were opened and mother liquor including the oligomer was added in aliquots to the droplet containing small initial crystals. In these cases, along with feeding, the MPD solution in the reservoir was replaced with MPD solution of a higher concentration, that is up to 65%(*v/v*).

3.3. *Preliminary analysis of crystals*

Many of the oligomers under investigation yielded crystals in more than only one condition of the matrix, opening the possibility of analyzing the influence of different cations on the crystal structure once high-resolution data were collected. Usually, after identifying the condition which produced the best crystal forms based on size and regularity, plates were prepared with 24 reproductions of this particular condition. In all cases, the results were highly reproducible and usually crystals grew in every droplet of the new plate. The crystals

Table 1. *Ingredients of the matrix*

The highest quality chemicals were purchased from Aldrich (Milwaukee, Wisconsin) or Sigma (St Louis, Missouri).

Stock solutions	
Buffers	Cacodylate buffer pH 7.0
	Cacodylate buffer pH 6.0
	Cacodylate buffer pH 5.5
Polyamines	Spermine tetrahydrochloride
	Cobalt hexammine chloride $Co(NH_3)_6Cl_3$
Monovalent ions	LiCl
	NaCl
	KCl
Divalent ions	$MgCl_2$
	$SrCl_2$
	$BaCl_2$
Precipitant	MPD (2-methyl-2,4-pentane-diol)

Table 2. *Composition of 24-condition matrix*

All conditions contained 40 m*M* buffer and 10%(*v/v*) precipitant (MPD); $SpCl_4$, spermine tetrachloride; $Co(NH_3)_6Cl_3$, cobalt-hexamminechloride.

Condition	pH	Polyamine	Monovalent ion	Divalent ion
1	7.0	12 m*M* $SpCl_4$	80 m*M* K^+	20 m*M* Mg^{2+}
2	7.0	12 m*M* $SpCl_4$	80 m*M* K^+	—
3	7.0	12 m*M* $SpCl_4$	80 m*M* Na^+	20 m*M* Mg^{2+}
4	7.0	12 m*M* $SpCl_4$	80 m*M* Na^+	—
5	7.0	12 m*M* $SpCl_4$	80 m*M* Na^+ 12 m*M* K^+	20 m*M* Mg^{2+}
6	7.0	12 m*M* $SpCl_4$	12 m*M* K^+ 80 m*M* K^+	—
7	6.0	12 m*M* $SpCl_4$	80 m*M* K^+	20 m*M* Mg^{2+}
8	6.0	12 m*M* $SpCl_4$	80 m*M* K^+	—
9	6.0	12 m*M* $SpCl_4$	80 m*M* Na^+	20 m*M* Mg^{2+}
10	6.0	12 m*M* $SpCl_4$	80 m*M* Na^+	—
11	6.0	12 m*M* $SpCl_4$	80 m*M* Na^+ 12 m*M* K^+	20 m*M* Mg^{2+}
12	6.0	12 m*M* $SpCl_4$	12 m*M* Na^+ 80 m*M* K^+	—
13	7.0	12 m*M* $SpCl_4$	80 m*M* Na^+	20 m*M* Ba^{2+}
14	7.0	12 m*M* $SpCl_4$	80 m*M* K^+	20 m*M* Ba^{2+}
15	6.0	12 m*M* $SpCl_4$	80 m*M* Na^+	20 m*M* Ba^{2+}
16	6.0	12 m*M* $SpCl_4$	80 m*M* K^+	20 m*M* Ba^{2+}
17	7.0	12 m*M* $SpCl_4$	40 m*M* Li^+	80 m*M* Sr^{2+} 20 m*M* Mg^{2+}
18	7.0	12 m*M* $SpCl_4$	40 m*M* Li^+	80 m*M* Sr^{2+}
19	7.0	12 m*M* $SpCl_4$	—	80 m*M* Sr^{2+} 20 m*M* Mg^{2+}
20	6.0	12 m*M* $SpCl_4$	—	80 m*M* Sr^{2+}
21	5.5	20 m*M* $Co(NH_3)_6Cl_3$	80 m*M* Na^+	20 m*M* Mg^{2+}
22	5.5	20 m*M* $Co(NH_3)_6Cl_3$	80 m*M* K^+	20 m*M* Mg^{2+}
23	5.5	20 m*M* $Co(NH_3)_6Cl_3$	12 m*M* Na^+ 80 m*M* K^+	—
24	5.5	20 m*M* $Co(NH_3)_6Cl_3$	40 m*M* Li^+	20 m*M* Mg^{2+}

Table 3. *Crystallized specimens*

^{Br}C, 5-bromo-cytosine; ^{F}U, 6-fluoro-uracil; ^{Br}U, 6-bromo-uracil; ^{I}U, 6-iodo-uracil.

Sequence	Conditions	Comments
DNA–drug complexes		
d(CGATCG)–*N,N*-(dimethyl)-adriamycin	1 to 12	$P4_12_12$ $a = 28.1$ $c = 53.4$ Å 1.7 Å resolution
d(CGATCG)–*N,N*-dimethyl)-daunorubicin	1 to 12	$P4_12_12$ $a = 28.1$ $c = 53.4$ Å 1.7 Å resolution
d(CGATCG)–4′-deoxy-4′-iododoxorubicin	1 to 24	$P4_12_12$ $a = 27.7$ $c = 52.5$ Å 1.5 Å resolution (Berger, Su *et al.*, 1995)
d(TGTACA)–4′-deoxy-4′-iododoxorubicin	2, 3	$P4_12_12$ $a = 27.9$ $c = 52.6$ Å 1.6 Å resolution (Berger, Su *et al.*, 1995)
d(TGTACA)–Pharmacia FCE23762*	14	$P4_12_12$ $a = 28.1$ $c = 52.9$ Å 1.7 Å resolution
d(GCCGGC)–*m*-amsacrine	16	Yellow diamond-shaped crystals, 0.5 mm diameter
d(TTGGCCA)–calicheamycin	17	Hexagonal plates, 0.8 mm diameter
d(GCG^{F}UACGC)–mithramycin A	4, 6	Colored monoclinic crystals, 0.2 × 0.3 × 0.8 mm
C-Tetrad motif		
d(CCCT)	19	$C222_1$ $a = 28.3$ $b = 44.3$ $c = 50.5$ Å 1.4 Å resolution (Kang *et al.*, 1994)
d(TCCCT)	10	Large hexagonal rods, 0.3 × 0.3 × 1 mm (disordered diffraction pattern beyond 2.5 Å resolution)
d(TCCC)	10	$P222$ 16 strands in the asymmetric unit
d(TAACCC)	9, 10, 13 to 16, 19, 21 to 24	$F222$ $a = 59.9$ $b = 81.3$ $c = 26.9$ Å 1.9 Å resolution (Kang *et al.*, 1995)
d(TAAC^{Br}CC)	9, 10	$F222$ $a = 59.0$ $b = 81.2$ $c = 26.8$ Å 1.7 Å resolution
d(TAA^{Br}CCC)	1 to 12, 19	Hexagonal, $a = 35.3$ $c = 51.1$ Å
d(CCCAAT)	1 to 12, 17, 18	$P62$ $a = 32.2$ $c = 52.5$ Å 2.0 Å resolution (Berger *et al.*, 1995)
d(CCCAA)	4	Small hexagonal rods
d(AAC^{Br}CC)	19, 20	Tetragonal rods
G-Quartet motif		
d(GGGGTTTTGGGG)	1, 2, 3, 4	$P2_12_12_1$ $a = 27.7$ $b = 49.6$ $c = 97.3$ Å 2.3 Å resolution (Kang, Zhang, Ratliff, Moyzis & Rich, 1992)
d(GGTTGGTTTGGTTGG)	13 to 16	Small bricks, 0.15 × 0.15 × 0.2 mm
d(GGGTTAGGG)	12	$P2_1$ $a = 33.3$ $b = 46.2$ $c = 33.4$ Å $\beta = 111.5°$ 2.5 Å resolution
d(TGGC)	11	$C222$ $a = 56.5$ $b = 56.2$ $c = 30.7$ Å $C222_1$ $a = 28.5$ $b = 54.6$ $c = 110.3$ Å
d(TGGGT)	3, 4, 9, 10	$P2_12_12_1$ $a = 55.7$ $b = 56.5$ $c = 28.5$ Å 1.5 Å resolution
d(TGGAGT)	1, 9	Flat tetragonal crystals, 0.25 × 0.25 × 0.4 mm
d(TGGAG^{I}UT)	3, 5, 7	Flat tetragonal crystals
d(TGGCGT)	2, 4, 6, 14	Hexagonal crystals, 0.2 × 0.2 × 0.3 mm
d(TGGGAT)	3, 4, 17	$I222$, $a = 57.2$ $b = 56.9$ $c = 70.1$ Å 1.5 Å resolution
d(^{Br}UGGGAT)	3, 4, 9, 12	Cubic crystals, 0.4 × 0.4 × 0.4 mm
RNA oligomers		
r(AUGUACAAAUAC)	4, 11	Tetragonal rods, 0.15 × 0.15 × 2.5 mm
r(UGGGU)	12	Diamond-shaped crystals, 0.4 mm diameter
r(GGGUCAGGG)	22, 24	Trapezoidal plates, 2.3 Å resolution
Others†		
d(CGCG)	1, 2, 4, 6, 10, 12	Large hexagonal rods
d(CGCGCG)	1 to 24	Large hexagonal rods and plates
d(GCGCGC)	10, 12	Diamond-shaped crystals, 0.8 mm diameter
d(CGCGCGCG)	1 to 12	Diamond-shaped crystals, 1 mm diameter
d(GCG^{F}UACGC)	1, 6	Monoclinic rods, 0.2 × 0.3 × 0.8 mm

* FCE23762, methoxymorpholino-doxorubicin. † The structures of $d(CG)_2$ $d(CG)_3$ and $d(CG)_4$ have been reported previously. These sequences were used to test the efficiency of the screen. As observed before, the crystals diffracted up to the limit of the Cu-sphere. No data sets were collected.

were analyzed for X-ray diffraction using a Rigaku R-AXIS IIc imaging plate (MSC, The Woodlands, TX, USA). Diffraction data of all crystallized specimens were collected under a 277 K nitrogen cold stream. Attempts to collect data at room temperature usually resulted in melting of the crystals, except for the crystals of drug–DNA complexes. Crystals of alternating purine–pyrimidine sequences all diffracted to better than 1.5 Å resolution as described previously in numerous publications. The guanine-rich, parallel tetraplex forming sequences up to a length of seven residues formed crystals which diffracted to 1.5 Å resolution, and no significant decay was observed over a period of 24 h of data collection. However, the longer sequences capable of forming looped antiparallel structures did not diffract that well and exhibited continuous decay from the start of the data collection. Crystals of sequences capable of forming four-stranded intercalated cytosine structures generally diffracted to beyond 2 Å resolution and did not exhibit any decay in up to 64 h of data collection. Table 3 lists preliminary data on some of the specimens under investigation. A detailed description of the refined structures will be provided elsewhere.

3.4. *Conclusions*

Crystal structure analysis of both DNA and RNA oligonucleotides of varying size and sequence provides a wealth of detailed information concerning the molecular geometry of the hereditary material and its messenger in the cell. If high-resolution diffraction data are available, X-ray crystallography makes possible a detailed investigation of molecular interactions, such as the binding of drugs to DNA as their target in the cell or the interactions between nucleic acid molecules and the ions and water molecules of its hydration shell. In recent years, numerous new structural motifs like the G-quartet or the four-stranded intercalated cytosine motif added to our knowledge of the polymorphism of nucleic acids in addition to the established A and B forms and Z-DNA. Consequently, a number of sparse-matrix crystallization screens have been developed, each with its own merits (Doudna, Grosshans, Gooding & Kundrot, 1993; Jancarik & Kim, 1994; Scott *et al.*, 1995). The 24-condition matrix method described here is fast and uses small amounts of sample, which makes possible a cost effective screening of many sequences and variations of a particular sequence. We have crystallized a large number of nucleic acid oligomers and the crystal structures are in various stages of refinement. We intend to make an effort to apply this matrix to larger molecules such as deoxy- and ribozymes, pseudoknots and tRNA's.

We thank Robert Moyzis and Robert Ratliff for the synthesis of many nucleic acid fragments; Thomas Burke for providing anticancer drugs and Li Su, April Fredian, and Wei-Hsiang Sun for assistance. IB is supported by a predoctoral fellowship from the Fonds der Chemischen Industrie (FCI, Germany). MW acknowledges support by the Deutsche Forschungsgemeinschaft (DFG, Germany).

References

Berger, I., Kang, C. H., Fredian, A., Ratliff, R., Moyzis, R. & Rich, A. (1995). *Nature Struct. Biol.* **2**, 416–425

Berger, I., Su, L., Spitzner, J. R., Kang, C. H., Burke, T. G. & Rich, A. (1995). *Nucleic Acids Res.* **23**, 4488–4494.

Chen, L., Cai, L., Zhang, X. & Rich, A. (1994). *Biochemistry,* **33**, 13540–13546.

Dickerson, R. E. (1991). *Hum. Genet.* **87**, 377–388.

Dickerson, R. E., Drew, H. R., Conner, B. N., Kopka, M. L. & Pjura, P. E. (1983). *Cold Spring Harbor Symp. Quant. Biol.* **47**, 13–24.

Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Fratini, A. V. & Kopka, M. L. (1982). *Science,* **216**, 475–485.

Dock-Bregeon, A. C. & Moras, D. (1991) *Crystallization of Nucleic Acids and Proteins, A Practical Approach*, edited by A. Ducruix & R. Giegé, pp. 145–174. Oxford: IRL Press.

Doudna, J. A., Grosshans, C., Gooding, A. & Kundrot, C. E. (1993). *Proc. Natl Acad. Sci. USA*, **90**, 7829–7833

Gehring, K., Leroy, J.-L. & Gueron, M. (1993). *Nature (London)*, **363**, 561–565.

Jancarik, J. & Kim, S. H. (1994). *J. Appl. Cryst.* **24**, 409–411.

Joshua-Tor, L. & Sussman, J. L. (1993). *Curr. Opin. Struct. Biol.* **3**, 323–335.

Kang, C. H., Berger, I., Lockshin, C., Ratliff, R., Moyzis, R. & Rich, A. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 11636–11640.

Kang, C. H., Berger, I., Lockshin, C., Ratliff, R., Moyzis, R. & Rich, A. (1995). *Proc. Natl Acad. Sci. USA*, **92,** 3874–3878

Kang, C., Zhang, X., Ratliff, R., Moyzis, R. & Rich, A. (1992). *Nature (London)*, **356**, 126–131.

Laughlan, G., Murchie, A. I., Norman, D. G., Moore, M. H., Moody, P. C., Lilley, D. M. & Luisi, B. (1994). *Science*, **265**, 520–524.

Leslie, A. G., Arnott, S., Chandrasegaran, R. & Ratliff, R. (1980). *J. Mol. Biol.* **143**, 49–71.

Scott, W. G., Finch, J. T., Grenfell, R., Fogg, J., Smith, T., Gait, M. J. & Klug, A. (1995). *J. Mol. Biol.* **250**, 327–332.

Wahl, M. C. & Sundaralingam, M. (1995). *Curr. Opin. Struct. Biol.* **5**, 282–295.

Wang, A. H.-J. & Gao, Y.-G. (1990). In *Methods: A Companion to Methods in Enzymology*, Vol. 1. New York: Academic Press.

Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G. & Rich, A. (1979). *Nature (London)*, **282**, 680–686.

Watson, J. D. & Crick, F. H. C. (1953). *Nature (London)*, **171**, 737–738.