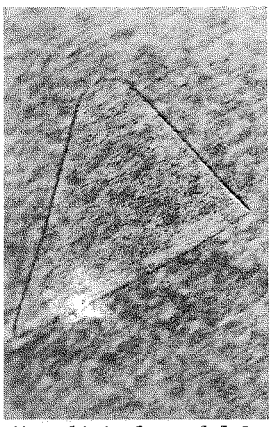
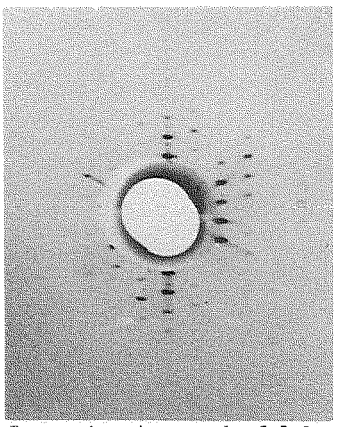
02.5–3 X-RAY DIFFRACTION AND ELECTRON-MICROSCOPIC STUDY OF 5 S rRNA CRYSTAL. By K. Morikawa, Department of Biophysics Faculty of Science, Kyoto University, Kyoto 606, Y. Fujiyoshi and K. Ishizuka, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan.

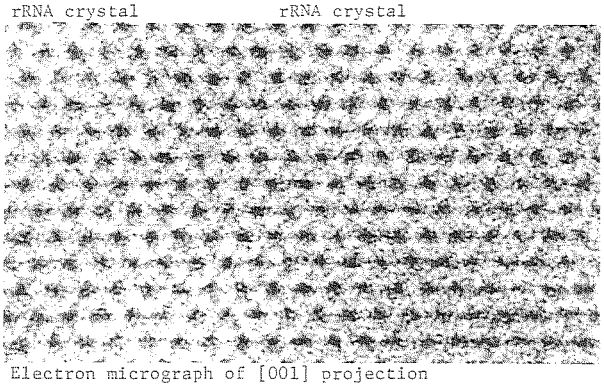
5 S rRNA is a common constituent that is present in the larger ribosomal subunit from prokaryotes to eukaryotes. For the first time we have succeeded in crystallization of 5 S rRNA from thermophilic bacteria, thrmus thermophilus HB8 (Morikawa et al., FEBS Letters (1982) 145, 194). 5 S rRNA is the second naturally occurring nucleic acid that has been obtained as a single crystal. We have subsequently been surveying crystallizing conditions to obtain crystals, which give higher order reflections good enough to construct a model at atomic resolution. To date, 5 different types of crystals have been grown by a vapour diffusion technique. An X-ray diffraction study of 3 types proved their crystal data to be (1) $P3_121$ ($P3_221$), a=b=99A, c=360A, 5 or 6 molecules/au(asymmetric unit) (2) P321, a=b=108A, c=133A, 2 molecules/au (3) P2 or 2_1, a=93A, b=55A, c=139A, $\beta=109^\circ$, 2 or 3 molecules/au. One of them was obviously cubic from observation under a polarised microscope. The last form of the crystals was thin enough to be subjected to electron microscopy. The crystal stained with uranyl acetate was thus found to preserve its regurality at 17A. The crystal data was found to be P321, a=b=90A, c=120A, 1 molecule/au from electron diffractions using a tilting stage. The subsequent image reconstruction study on [001] and [1$\bar{1}$2] projections revealed the molecular arrangements within the crystal lattice, and also consequently gave some information on the overall shape of 5 S rRNA molecules.



Monoclinic form of 5 S rRNA crystal



Precession photograph of 5 S rRNA crystal



Electron micrograph of [001] projection

02.5–4 CRYSTAL STRUCTURE OF THE LEFT-HANDED HELICAL RNA OLIGOMERS. BY Ken-ichi Tomita, Yumi Nakamura, Akira Nishimura, Satoshi Fujii, Seiichi Uesugi and Morio Ikehara, Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565, Japan.

The torsion angle of nucleotide, especially the glycosyl torsion angle, is the fundamental and most substantial factor to specify the helical sense of polynucleotide. Since the discovery of the left-handed Z-DNA structure, many attempts to elucidate its biological significance have been made. On the other hand, it is still unknown whether the double-stranded RNA is also possible to form the Z-like left-handed double helical structure.

In this report, we describe the crystal structure analysis of two oligo-ribonucleotides designed in such a way that the oligomer has the self-complementary and C-G alternating sequence and guanosine residue tends to form the syn-conformation because of the bulky substitution at the C-8 position.

The chemically synthesized RNA fragment, $r(C\text{-}br^8G)_2$ or $r(C\text{-}m^8G)_3$, was crystallized in a solution containing oligomer, sodium cacodylate buffer(pH 7.0) and magnesium chloride(in case of need, spermine) by vapor diffusion from 10 to 30%(v/v) 2-methyl-2,4-pentanediol. The crystallographic data are:

$r(C\text{-}br^8G)_2$: hexagonal, space group $P6_5$, a=b=32.05, c=42.07Å, $\gamma=120^\circ$.

$r(C\text{-}m^8G)_3$: Hexagonal, space group $P6_5$, a=b=18.41, c=42.63Å, $\gamma=120^\circ$.

Three-dimensional data for both crystals were collected to a resolution of 1.5Å by using a Rigaku four-circle diffractometer. The cell dimensions and the intensity distribution of (0kl) reflections of $r(C\text{-}br^8G)_2$ crystal are very similar to those of DNA fragmnet $d(C\text{-}G)_2$ (J.L.Crawford et al., Proc.Natl.Acad.Sci.USA., 77, 4016 (1980)) and the a- and b-axes of $r(C\text{-}m^8G)_3$ were obtained by rotating the hexagonal c-axis of $d(C\text{-}G)_2$ crystal by 30°. These facts may indicate that the RNA oligomers, $r(C\text{-}br^8G)_2$ and $r(C\text{-}m^8G)_3$, should form left-handed double helical structures and, therfore, we tried to solve the structure by using the atomic coordinates of $d(C\text{-}G)_2$ hexagonal crystal as the starting left-handed RNA model, and refine the structure by using the Konnert-Hendrickson refinement procedure. At the present stage, the R-factors are 28% at 2.0Å for $r(C\text{-}br^8G)_2$, and 22% at 1.8Å for $r(C\text{-}m^8G)_3$, respectively. Even the positions and numbers of solvent molecules involved in the unit cell and also the effect of syn-conformation on the sugar puckering of guanosine moieties are still not clear, we became convinced that these RNA oligomers in the crystalline state form the left-handed double helical structure which is similar but not identical to the Z-form found in DNA fragment.