Crystals of Ribosomes, Exhibiting Severe Non-isomorphism, Extreme Radiation Sensitivity, and No Internal Symmetry, as Subjects for Synchrotron Radiation Crystallography

Ada Yonath
Weizmann Institute, Rehovot and Max-Planck-Research-Unit, Hamburg, Israel

In rapidly growing bacterial cells, the translation of the genetic code into polypeptide chains consumes up to 80% of the cell's energy and constitutes about half of its dry weight. This fundamental life process is performed by more than a hundred components, among which are giant nucleoprotein assemblies called ribosomes, the universal organelles facilitating the sequential polymerization of amino acids according to the blueprint encoded in the mRNA. Bacterial ribosomes (M.W. 2.3 mD) are built of two independent subunits of unequal size which associate upon the initiation of protein biosynthesis. The large subunit (1.45 mD) catalyzes the formation of the peptide bond and provides the progression path of the nascent proteins. The small subunit (0.85 mD) contains the site for the initiation of the process and for the decoding of the genetic information. About one third of the ribosomal mass comprises some 58-73 different proteins, depending on its source. The remaining two thirds are three chains of RNA, a total of about 4500 nucleotides.

Crystals have been grown from intact ribosomes and their subunits, despite their unfavorable properties (enormous size, lack of internal symmetry, inherent flexibility, and a surface composed of highly degradable RNA with proteins which may be loosely held). Far beyond the initial expectations, two crystal types, from the large ribosomal subunits of *Haloarcula marismortui* (H50S) and from the small subunit of *Thermus thermophilus* (T30S), diffract to around 0.3 nm. However, high resolution is not necessarily linked to high quality diffraction. On the contrary, the crystal type diffracting to the highest resolution (H50S), yields the most problematic diffraction data.

The bright synchrotron radiation x-ray beam, necessary for the collection of the high-resolution x-ray diffraction data, causes significant decay even at cryo temperature. Nevertheless, due to the reasonable isomorphism of the T30S crystals, reliable MIR phases were determined. The resulting 0.55-nm electron-density map contains features which can be interpreted as ribosomal proteins as well as long continuous chains that were traced as the RNA double helices, loop regions and single strands. In contrast, the substantial radiation sensitivity of H50S is accompanied by a low level of isomorphism, instability of the unit cell dimensions, low reproducibility, deformed spot-shape, and non-isotropic mosaicity. The 0.85-nm MIR electron density map, constructed to gain insight into this unusual system, may indicate the reasons for the problematic nature of the H50S crystals and provide hints for their improvement.

The progress of ribosomal crystallography (including anomalous and MAD phasing), as well as molecular replacement studies, and the exploitation of images reconstructed from electron micrographs of ribosomal particles embedded in vitreous ice, will be discussed.