The Discovery of Z-DNA: the Work of Alexander Rich

The Effect of Anti-Z-DNA Antibodies on the B-DNA-Z-DNA Equilibrium

Alexander Rich was born in Hartford, Connecticut in 1924. He enrolled at Harvard College in 1942 and shortly thereafter enlisted in the Navy V12 Program, which he completed in 1944. He was then sent to work in a hospital at a submarine base and then at Syracuse Medical School. Upon being discharged from the Navy in January 1946, he returned to Harvard College to complete his undergraduate degree and to start a research program. Working with Journal of Biological Chemistry (JBC) Classic author John T. Edsall (1), Rich studied several amino acids and dipeptides using Raman spectroscopy and identified the Raman bands associated with stretching vibrations of the peptide bond. He also carried out an infrared spectral analysis of urea with E. Bright Wilson.

Upon earning his undergraduate degree, Rich faced a dilemma. He was interested in pursuing the physical chemistry of biological materials, but at the same time, he thought that medical school would be able to give him a detailed view of the biology of one organism. After much debate, he decided to complete his last 2 years of medical school at Harvard while continuing to work with Edsall.

Rich earned his M.D. in 1949 and decided to go directly into research rather than do an internship. He was accepted into JBC Classic author Linus Pauling’s (2) laboratory at the California Institute of Technology. Motivated by Pauling, who stated that knowing the structure of molecules led to their properties and function, Rich switched his research to x-ray structural analysis and solved the structure of ferrocene (3). For a brief period, Rich also tried to obtain x-ray diffraction patterns of DNA, but after hearing that Watson and Crick had solved the structure, he turned his attention to determining whether or not RNA could also form a double helix.

In 1952, Rich enlisted in the U. S. Public Health Service and was appointed Chief of the Section on Physical Chemistry at the newly formed National Institute of Mental Health. Because his future laboratory was still being built, Rich remained at Caltech for 2 more years before leaving for Bethesda.

After finally starting his independent research program, Rich was determined to continue his work on RNA structure and the question of whether it could form a double helix. He eventually did both of these, discovering the structure of an adenine and uracil copolymer that produced a diffraction pattern very similar to that of RNA (4) and showing that, when mixed together, solutions of polyriboadenylic acid (poly(rA)) and polyribouridylic acid (poly(rU)) reacted to produce a double helix (5).
In 1958, Rich left the NIH and moved to the biology department at the Massachusetts Institute of Technology. There he shifted the focus of his research and began looking at the mechanism of protein synthesis. In 1962, he published a paper demonstrating that protein synthesis occurred in a cluster of ribosomal particles, which he called the “polyribosome” or “polysome” (6). He followed this by showing that there are two tRNA-binding sites in the ribosome: Site A, which bound aminoacyl-tRNA, and Site P, which bound peptidyl-tRNA. He postulated that the two sites acted in a coordinated manner to transfer the growing polypeptide chain and to move the mRNA codon from Site A to Site P. Finally, in 1973, he determined the structure of yeast tRNA^{Phe} (7). The backbone tracing of this molecule was published on the front page of The New York Times on January 13, 1973, together with a discussion of its role in protein synthesis.

In 1979, Rich and his colleagues solved the structure of a self-complementary DNA hexamer d(CG)₃. To their surprise, they discovered that the molecule formed a left-handed double helix with two antiparallel chains held together by Watson-Crick base pairs (8). Every other base in this new helix was rotated around the glycosyl bonds so that the bases alternated in anti and syn conformations along the chain. This caused a zigzag arrangement of the backbone of the molecule, affording it the name “Z-DNA.”

A relationship between Z-DNA and B-DNA was hinted at by Pohl and Jovin in 1972 when they showed that the ultraviolet circular dichroism of poly(dG-dC) was nearly inverted in 4 M sodium chloride solution (9). Rich confirmed that this was the result of a conversion from B-DNA to Z-DNA by examining the Raman spectra of Z-DNA crystals in similar solutions (10). It soon became apparent that the ground state of DNA in a physiological solution was B-DNA and that the Z conformation was a higher energy state. Rich also showed that negative supercoiling could stabilize Z-DNA (11).

However, after several more years of research turned up nothing definitive about the biological role of Z-DNA, many scientists lost interest in the helix, believing that Z-DNA was a nonfunctional conformational phenomenon. Rich, on the other hand, was convinced that Z-DNA had a biological role. The first indications of this role came from immunological work showing that Z-DNA, unlike B-DNA, is highly antigenic and yields polyclonal and monoclonal antibodies.

These antibodies proved to be a useful tool in characterizing chromosome organization and the B-DNA-Z-DNA equilibrium. However, the question soon arose as to whether the presence of the antibodies could influence the appearance of Z-DNA. Rich decided to look at how the free energy of antibody binding affects the B-Z equilibrium for both the salt-induced and supercoiling-induced B-Z transitions. This is the subject of the JBC Classic reprinted here.

Using four different preparations of rabbit and goat anti-Z-DNA antibodies, Rich and his colleagues showed that the antibodies did cause a marked lowering in the ionic strength required for the B-DNA to Z-DNA transition and that the presence of anti-Z-DNA antibodies reduced the degree of negative supercoiling required for the formation of Z-DNA. Thus, Rich concluded, “The stabilization of Z-DNA by antibody binding in a supercoiled plasmid can be significant, and failure to consider this effect and to choose appropriate conditions for measurement can lead to errors in estimating when Z-DNA will form in response to negative supercoiling.”

Investigations into the biological role of Z-DNA continued, and it was eventually discovered that Z-DNA is largely found in transcribing genes and that the cessation of transcription results in rapid conversion of Z-DNA to B-DNA through the action of topoisomerases. Rich proposed that the negative torsional strain induced by the movement of RNA polymerase stabilized Z-DNA formation near the transcription start site. Thus, Z-DNA was a metastable conformation, forming and disappearing depending upon physiological activities.

Rich remains at MIT as the William Thompson Sedgwick Professor of Biophysics. He also founded the biotechnology company Alkermes in 1987, along with Michael Wall, Floyd Bloom, and Paul Schimmel. He is a member of the National Academy of Sciences, the American Philosophical Society, the Pontifical Academy of Sciences, Rome, and a foreign member of the French Academy of Sciences, Paris. Rich’s outstanding scientific achievements in science were recognized by President Clinton when he was awarded the Medal of Science in 1995. His other awards include the 2001 William Procter Prize for Scientific Achievement and the 2008 Welch Award in Chemistry.¹

Nicole Kresge, Robert D. Simoni, and Robert L. Hill

¹ Biographical information for this article was taken from Refs. 12 and 13.
REFERENCES


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