David S. Woodruff
Department of Biology,
University of California, San Diego,
La Jolla, CA 92093–0116, USA

■

The Theory of DNA Bending

A. D. Mirzabekov and A. Rich (*1*) conjectured in 1979 that charge neutralization of phosphate groups along one side of a DNA segment could cause the DNA to bend toward the neutralized side. Ten years later, this reasonable idea was finally analyzed with the tools of polyelectrolyte and elasticity theory (*2*), and it was concluded that even low degrees of unilateral phosphate neutralization would be sufficient to bend DNA to a structurally significant extent. The conjecture and supporting theory were recently confirmed experimentally by Juliane K. Strauss and L. James Maher III in their Research Article "DNA bending by asymmetric phosphate neutralization" (16 Dec., p. 1829), which was also discussed in an accompanying Perspective by D. M. Crothers (p. 1819). The experimental data are reported to be in general agreement with the predictions of the theory. Strauss and Maher note, however, that one of the quantitative predictions of the theory is not observed. The theory predicts that the radius of curvature of the bend depends on the length of the DNA segment. The observation is that the radius is the same over the length range studied.

The discrepancy is only apparent. The DNA molecules synthesized by Strauss and Maher possess discrete "patches" six base pairs long, completely neutralized on one side. DNA molecules of different lengths contain more patches, but the bending is localized to each patch. The radius of curvature is the radius characterizing the bent six–base pair neutral patch, regardless of the overall length of the DNA within which the patches are embedded.

The theoretical equations are applicable to the DNA segment that is unilaterally neutralized. In this case they are applicable to the six–base pair patch completely neutralized along one side. I have set the length parameter L in the theory equal to the length of six base pairs of DNA. I have also set the fractional extent of unilateral charge neutralization α equal to unity. In univalent buffer the theoretical formula then predicts that the bending angle is about 9°. The value of the bending angle in tris buffer measured by Strauss and Maher is about 21°. The list of reasons not to expect better than factor of 2 agreement between theory and experiment is long. Perhaps the most obvious is the almost complete lack of molecular-structural detail in the theoretical model.

Gerald S. Manning
Department of Chemistry,
Rutgers University,
New Brunswick, NJ 08903, USA

References

1. A. D. Mirzabekov and A. Rich, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 1118 (1979).
2. G. S. Manning, K. K. Ebralidse, A. D. Mirzabekov, A. Rich, *J. Biomol. Struct. Dynam.* **6**, 877 (1989).

■

Biotech Patents and "Usefulness"

The article by Richard Stone "Rules would drop need for clinical data" (News, 6 Jan., p. 23) could leave the reader with a misleading impression about guidelines proposed by the U.S. Patent and Trademark Office (PTO) with respect to the standard to which patent applicants are held in substantiating the "usefulness" of an invention. Far from being a "significant concession" to the demands of the biotechnology industry, the PTO guidelines provide a road map to help patent examiners to apply what has been long-settled law in this area.

The clinical data which PTO examiners have sought from inventors were not merely "unrealistic," but also were not required by law. Case law established over many years mandates that the PTO must accept an inventor's assertion of a utility for an invention unless a reasonable, scientific basis exists to doubt that assertion.

In the course of analyzing patent applications in the biotechnology area, many examiners stood this principle on its head by presuming therapeutic inventions to be "incredible" unless proven otherwise; this, despite the fact that the category of "incredible" inventions had been reserved for perpetual motion machines, engines that run on tapwater, and the like. Applicants then were subjected to what many felt were unreasonable demands for evidence, including human clinical data, to prove that the invention was useful in a practical sense.

According to the proposed guidelines, by contrast, examiners of biotechnology applications are to consider the utility of a claimed invention in conformance with established U.S. patent law practice. The guidelines (*1*) state, for example, that examiners should consider whether a patent applicant "has asserted that the *claimed* invention is useful for any particular purpose and that assertion would be consid-

ered *credible* by a person of ordinary skill in the art" (emphasis in the original). In addition, the guidelines counsel examiners to accept an expert's credible statements that support patentability unless the examiner "can show that one of ordinary skill in the art would have a rational basis to doubt the truth of such statements." In legal effect this means that the examiner must show that such a one would be more likely to disbelieve than to believe the asserted utility.

Commissioner Bruce Lehman is to be congratulated for taking prompt action to ensure that biotechnology patent applications will now be judged by the same legal standards of utility that are applied to patent applications in other technical fields.

Stephen A. Bent
Paul M. Booth
Karen Kaechele Costantino
Phillip B. C. Jones
Gary L. Shaffer
Melvin Blecher
John P. Isacson
Richard C. Peet
Foley and Lardner, Biotechnology and Pharmaceutical Practice Group, Washington Harbor, Suite 500, 3000 K Street, NW, Washington, DC 20007–5300, USA

References

1. *Fed. Regist.* **60**, 97 (3 January 1995).

■

Low-Barrier Hydrogen Bonds

In our report of 24 June 1994 (p. 1927), Sean A. Whitt, John B. Tobin, and I (*1*) described the spectroscopic evidence implicating a low-barrier hydrogen bond (LBHB) linking $N^{\delta 1}$ of His^{57} with the β-carboxyl group of Asp^{102} in the protonated triad of chymotrypsin. We further described a first approximation to a chemical model for this LBHB. After noting the spectroscopic evidence for an LBHB in a close model of the tetrahedral intermediate in chymotrypsin (*2*), we postulated a mechanism in which the LBHB linking the dyad His^{57} and Asp^{102} facilitates the nucleophilic addition of the β-OH group of Ser^{195} to a substrate by stabilizing the dyad on the enzymatic side of the tetrahedral intermediate. To the extent that the transition state resembles the intermediate, its energy would be lowered by this interaction and the rate would be enhanced.

Richard L. Schowen has pointed out that he and his associates earlier suggested the participation of strong hydrogen bonds in "catalytic proton bridges" that facilitate multiproton catalysis by serine proteases [3 (p. 119), *4*]. It is true that Showen and his colleagues speculated on the possible importance of "Kreevoy-type" hydrogen bonds in multiproton catalysis. Their suggestions differed from our postulate, however, in several respects. First, they emphasized the possibility that strong hydrogen bonds in putative hydrogen bonding networks might stabilize the transition state as distinguished from the intermediates. Second, they consistently suggested the simultaneous participation of more than one strong hydrogen bond. They did not specify the positions of strong hydrogen bonds, but included the hydrogen bond bridging Asp^{102} and His^{57} in the putative network. Third, their suggestions were inspired by the observation of small isotope effects, which are consistent with, but do not specifically implicate, strong hydrogen bonds.

In this connection, G. Robillard and R. G. Shulman, who described the low-field proton in the catalytic triad and assigned it to $His^{57}(N^{\delta 1})$–Asp^{102} (*5*), later suggested that a hydrogen bond linking $His^{57}(N^{\epsilon 2})$ with the leaving group in a tetrahedral intermediate might be characterized by a single minimum potential, that is, it might be a single-well hydrogen bond (*6*). This suggestion arose from studies of boronate inhibitors. Our postulate is not related to this latter suggestion.

The main purpose of our report was to point out that LBHB's are characterized by spectroscopic signatures, including a low-field nuclear magnetic resonance chemical shift; that a low-field proton appears in the protonated catalytic triad, as well as in a tetrahedral adduct of chymotrypsin with *N*-acetyl-L-Leu-L-Phe trifluoromethylketone and in a simple model for the dyad; that the low-field proton in chymotrypsin exhibits a deuterium isotope effect that is characteristic of protons participating in low-barrier hydrogen bonds; and that the participation of this low-barrier hydrogen bond in catalysis can resolve a long-standing controversy regarding the mechanism of action of serine proteases.

Perry A. Frey
Institute for Enzyme Research, University of Wisconsin, Madison, WI 53705–4098, USA

References

1. P. A. Frey, S. A. Whitt, J. B. Tobin, *Science* **264**, 1927 (1994).
2. T.-C. Liang and R. H. Abeles, *Biochemistry* **26**, 7603 (1987).
3. R. L. Schowen in *Mechanistic Principles of Enzyme Activity*, J. F. Liebman and A. Greenberg, Eds. (VCH, New York, 1988), pp. 119–168.
4. R. L. Stein, J. P. Elrod, R. L. Schowen, *J. Am. Chem. Soc.* **105**, 2446 (1983).
5. G. Robillard and R. G. Shulman, *J. Mol. Biol.* **71**, 507 (1972).
6. ———, *ibid.* **86**, 541 (1974).