The name is bond — H bond

T.W. Martin and Zygmunt S. Derewenda

The hydrogen bond plays a critical role in diverse biological phenomena. Although discovered 90 years ago, the precise chemical nature of this unique interaction has remained in dispute. A recent Compton-scattering experiment, however, strongly supports a partially covalent picture of the hydrogen bond.

"It seems to me that the most important addition to my theory of valence lies in the suggestion of what has become known as the hydrogen bond."

Gilbert N. Lewis, *Valence and the structure of atoms and molecules*, 1923.

"I believe, that as the methods of structural chemistry are further applied to physiological problems it will be found that the significance of the hydrogen bond for physiology is greater than that of any other single structural feature."

Linus Pauling, *The nature of the chemical bond*, 1939.

Hydrogen bonds constitute a unique type of interatomic interaction, with energies typically in the range of 2–10 kcal mol^{-1}. These values are intermediate between covalent bonds and weak interactions, such as Van der Waals forces. The small but significant energy of the hydrogen bond allows its formation and disruption under a wide range of physiological temperatures and conditions. Hydrogen bonds are directional and are therefore suited to play a role in molecular recognition phenomena. Moreover, most enzymatic reactions rely on shuttling protons, very often utilizing networks of hydrogen bonds. Given the obvious importance and ubiquitous nature of hydrogen bonds, it is remarkable that their chemical character has often been fiercely debated in the literature. However, the debates should now subside, thanks to a recent publication in *Physical Review Letters*[1]. A group of researchers at Bell Laboratories/Lucent Technologies and their French (ESRF) and Canadian (NRC) colleagues describe a Compton-scattering experiment that unambiguously demonstrates that hydrogen bonds are partly covalent, thus ending decades of controversy.

It is not easy to trace the history of the hydrogen bond concept, particularly since the term was not introduced until the 1920s. The idea was conceived at the Chemical Laboratory of the University of California, Berkeley, already renowned for the work of G.N. Lewis who formulated the valence theory in 1916. Apparently, the concept was developed independently by Maurice Huggins, who used it in a thesis to explain tautomerism in acetoacetic acid esters, and by Wendell Latimer and Worth Rodebush[2], who are typically credited with the first publication on the subject. In 1922, Linus Pauling arrived in California, albeit at Pasadena's California Institute of Technology rather than his first choice, Berkeley. He read the paper by Latimer and Rodebush soon after, and the concept became a favorite of his. After spending some time in Germany with Arthur Sommerfeld, Pauling returned to Pasadena where, as a faculty member, he resumed work on the nature of the chemical bond and published his first paper addressing the hydrogen bond in 1928[3].

The hydrogen bond concept was not widely known for the first decade of its existence. In 1933, J.D. Bernal and R. Fowler published their seminal paper on the structure of water[4], inspired, according to Ilya Ehrenburg, by a rainy day at the Moscow central airport as both scientists waited under an awning[5]. Although the paper discusses at length the tetrahedral coordination of water molecules, nowhere are hydrogen bonds mentioned by name, nor are references to Latimer and Rodebush or Pauling to be found. Similarly, the concept may not have been known to William Astbury, who published his models of α- and β-keratin that same year[6]. Astbury referred only to 'attractions' between NH and CO groups in adjacent polypeptide chains, which he visualized in the diagrams with broken lines identical to those with which he linked adjacent CαHR groups (Fig. 1*a*). Reading these two papers one cannot escape the conclusion that English science did not have hydrogen bonds in its vocabulary in 1933.

This situation changed a year later when Bernal completed a treatise on the function of hydrogen in intermolecular forces, explicitly invoking the hydrogen bond (curiously phrased "the so-called hydrogen bond of Huggins and Pauling", with no reference to Latimer and Rosebush) and proposing — unnecessarily — a new type of this interaction, the 'hydroxyl bond', in which two hydroxyls are bonded by two symmetric hydrogen bonds[7]. In the same year, the structure of water was addressed by Pauling, who pointed out that many properties of water and ice can be attributed to hydrogen bonds[8].

The structure of proteins was also quickly revisited. In their paper on the denaturation of proteins, Alfred Mirsky and Pauling suggested that the conformation of a polypeptide chain is defined by hydrogen bonds between the peptide nitrogen and oxygen atoms, although they did not show any structural details[9]. This was the first instance of hydrogen bonding explicitly implicated in protein structure. Shortly after, and

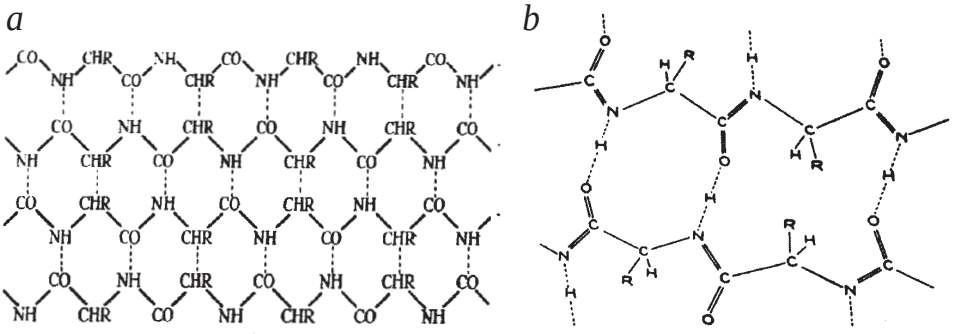

Fig. 1 Early views of interactions of amide and carbonyl groups in proteins. ***a***, Astbury's model of keratin in 1933 (adapted from ref. 6). ***b***, Huggins' description of inter-chain hydrogen bridges in 1937 (ref. 10).

probably before Pauling's paper was published, Huggins[10] carefully analyzed Astbury's diagrams, and pointed out that the amide hydrogen must be out of the plane of the peptide (the entire polypeptide backbone was assumed to lie in one plane) unless resonance is invoked, so that "the orbital of the lone pair on the nitrogen (is) in the plane of the chain" (Fig. 1*b*). Sadly, Huggins failed to realize the importance of his statement, and did not expand on the consequences of resonance in the peptide bond. Nonetheless, he bitterly argued for the rest of his career that it was he, and not Pauling, who first proposed that the peptide unit is planar[11].

As the American chemists were converging on protein structure, in England, Dorothy Wrinch was advocating the 'cyclol' theory of proteins[12], in which the peptide linkages took the form of -C(OH)-N = instead of -(CO)-(NH)-, as others were proposing. In this theory, no hydrogen bonds of the classical type were possible. Pauling was quick to recognize the faults in this model and published a critical paper in July of 1939, in which he emphasized the planarity of the peptide bond[13]. His paper was out only weeks before Nazi Germany invaded Poland on September 1, and it was very likely missed by many European scientists. That same year, Pauling published his textbook *The nature of the chemical bond*, which devoted a full chapter to hydrogen bonding.

With the war on, the study of hydrogen bonds and proteins was not a top priority. One of the few noteworthy papers of those years was authored, again, by Huggins, who took his ideas on protein conformation further and proposed parallel and antiparallel extended sheets and helical structure[14]. As already noted, he failed to grasp the importance of the planarity of the peptide bond in the helices, although one of his models is very close to a 3_{10}-helix. The sheets also contain errors, although the hydrogen-bond pairing is essentially correct. Interestingly, Huggins also implicates the Cα-bound protons in hydrogen bonds with carbonyl oxygens in the adjacent chains. This was the first-ever reference to C-H·····O hydrogen bonds in proteins, an idea that took over 50 years to resurface but which is now generally accepted[15,16].

The more recent history of the hydrogen bond concept in structural biology is better known. In 1951, Pauling, Robert Corey and H. Branson published accurate models of helical and extended polypeptide chains, and two years later Crick and Watson used hydrogen bonding between bases in their DNA model to achieve complementarity. In 1957 a major international conference, sponsored by IUPAC, was convened in Yugoslavia to discuss the progress in the understanding of the hydrogen bond. Papers were presented by Pauling, Bernal, Pople, Coulson, Eigen and others.

In spite of substantial progress, the problem of the exact nature of the hydrogen bond remained unsolved. Early on (contrary to some literature) Pauling and others considered the hydrogen bond to be essentially an electrostatic interaction. It was not until later that Pauling proposed the partially covalent character of this interaction. Although there is always some theoretical overlap of electronic wavefunctions between neighboring atoms, Pauling was suggesting that electron sharing might be energetically significant enough to explain many hydrogen bond features, such as its strength and directionality. Chemical evidence, unfortunately, was indirect and ambiguous and the notion has been disputed ever since. The experimental validation of Pauling's idea had to await the results of a 23-hour X-ray scattering experiment performed at the ESRF in Grenoble last year[1]. Analysis of these data has provided the first 'Compton profile' of the hydrogen bond wavefunction in Ice Ih (which is common ice, space group $P6_3/mmm$).

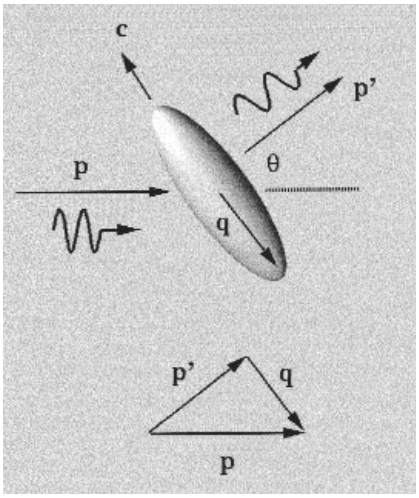

Fig. 2 Compton scattering from an anisotropic wavefunction. An incoming photon with momentum **p** and wavelength λ will always scatter with a smaller momentum **p'** and a longer wavelength. The scattering angle is θ. The vector c represents the direction of the bond axis, while **q** represents the momentum transferred to the recoiling electron. The momentum change of the photon is equal and opposite to that of the electron, since momentum conservation requires **p** = **p'** + **q**. In the illustration, a photon scatters off one of the allowed electron momentum states of the wavefunction, which is necessarily oriented along the c axis. Essentially no scattering comes from covalent states when the bond axis c, and a potential scattering vector **q** are perpendicular.

A brief review of the method is in order. Thomson scattering, which forms the basis of standard X-ray crystallography, can be conceptualized in essentially classical terms: an incoming electromagnetic wave induces point particles to vibrate, so that these in turn become sources of radiation with wavelength identical to that of the incident ray. These waves can then interfere and produce diffraction-like effects in the familiar manner. Although an obvious oversimplification, this picture is quite useful in practice. Compton scattering, in contrast, is a fundamentally quantum-mechanical phenomenon. Here we imagine the scattering event to be the collision of a photon with another particle, such as an electron. The scattered photon loses energy and momentum to the particle, and therefore experiences a change in wavelength.

Arthur Compton used simple energy/momentum conservation to derive a relation between photon wavelength shift and scattering angle: $\Delta\lambda = h(1 - \cos\theta)/mc$. (where $\Delta\lambda$ is the wavelength shift, θ is the scattering angle, m is the mass of the particle, h is Planck's constant and c is the speed of light)[17]. He verified this result in 1922 and received a Nobel prize for this work in 1927. The form of the equation used in the present experiment is written in terms of energy shift: $\Delta E = h^2q^2/2m + hq.p_e/m$ (where q is the magnitude of **q**, that is, $q=|\mathbf{q}|$, q is the momentum transferred to the electron and p_e is the electron momentum itself). The first term corresponds to Compton's original equation, while the second is a 'Doppler' portion. The second term enables researchers to probe the momentum states of a system by recording the intensity (number) of photons deflected at various angles. For an electron, the set of allowed momentum states corresponds to its probability density (wavefunction). The more prominent a given momentum state is in a wavefunction, then the greater the number of photons scattering with that momentum shift. Such a pattern of intensity *versus* scattering angle constitutes the Compton profile of the system.

Thomson scattering yields its strongest signal from tightly-bound core electrons, insofar as these electrons elastically 'reflect' the incoming wave. Indeed, Thomson scattering is just a special case in which $\Delta\lambda$ is negligible because m in the above equation is effectively the mass of the atom, rather than the electron. For this reason, Compton scattering is better suited to the detection of valence electrons. Outer shell electrons are held comparatively weakly, and therefore recoil more like free electrons. In particular, electrons within covalent

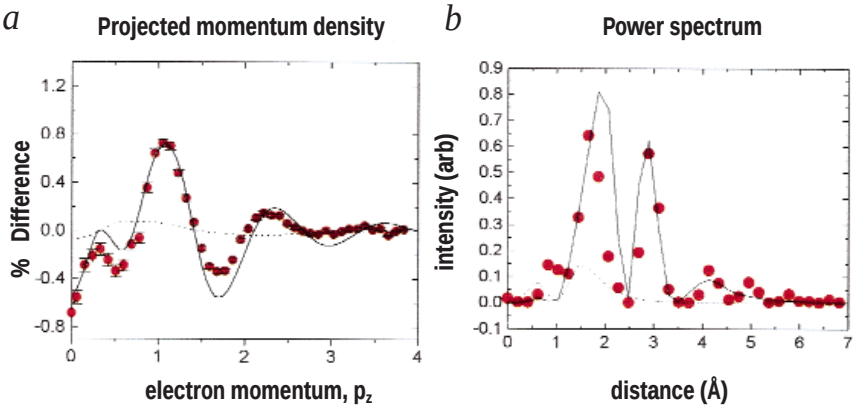

Fig. 3 ***a***, Electron momentum *versus* anisotropy. This is the Compton profile of the electronic wavefunction with the isotropic component subtracted out. The dots represent the measured data values. The solid line depicts the expected profile based on a quantum-mechanical model of the hydrogen bond, while the dotted line depicts the profile expected using a classic, purely electrostatic picture of the hydrogen bond in water. ***b***, This is the Fourier transform of (*a*) from momentum-space to position-space. The key to the lines is the same as in (*a*). The peaks at 0.89, 1.72 and 2.85 Å indicate the positional bounds of the momentum states—that is, they indicate the lengths of covalent wavefunctions. Peaks at higher values are simply extensions of these continuous wavefunctions in the crystal lattice. The 2.85 Å peak indicates an extended wavefunction between nearest-neighbor oxygens (O-O). Note that the hydrogen bond peak (1.72 Å) and the O-O peak are strong because they represent electrons that are held more loosely, and which therefore act as strong Compton scatterers. The O-H bond (0.89 Å) has a very weak peak by comparison. The 'classic' trace using an electrostatic prediction (dotted line) has a small peak at ~1.0 Å because it contains the single, strong covalent bond between oxygen and hydrogen. Figures modified from ref. 1, courtesy of E. Isaacs.

bonds have highly directional wavefunctions, and therefore have momentum states that tend to be directed along a specific axis. As a consequence, the Compton scattering profile of a covalent bond depends strongly on the relative orientation of the scattering vector and the bond axis (Fig. 2).

The Grenoble experiment hinges on this anisotropy. Two trials were performed: one with the crystal placed so that the scattering vector q was aligned with the hexagonal c-axis (itself aligned with a high proportion of hydrogen bonds), and one with the scattering vector aligned perpendicular to that axis. The values of photon momenta at a range of angles were recorded for both trials. The initial data take the form of an electron momentum density, because for each detected (scattered) photon momentum, an electron momentum is determined, and the percent difference between the two trials depicted (Fig. 3*a*). This procedure effectively subtracts out isotropic scattering, such as that produced by inner shell electrons, as well as background from bonds not oriented along the axis. In principle, only those extended wavefunctions oriented along the c-axis should stand out.

The oscillatory behavior illustrated in Fig. 3*a* arises from interference generated by overlapping wavefunctions — that is, from covalent bonds. This momentum-space profile of the net wavefunction can then be Fourier-transformed into a position-space profile, generating a graph of intensity *versus* length (Fig. 3*b*). 'Length' corresponds to the bounds of the component wavefunctions. Peaks at ~0.89, 2.85 and 1.72 Å correspond closely to the O-H bond (1.0 Å), nearest-neighbor O-O distance (2.75 Å), and hydrogen-bond distance (1.75 Å), respectively. These last two peaks represent the significant new experimental result. They are direct evidence for a continuous, phase-coherent wavefunction between nearest-neighbor oxygen atoms in the ice lattice, with part of the wavefunction composed of the hydrogen bond itself (Fig. 4). The electrostatic aspect of the hydrogen bond must therefore be understood in conjunction with wavefunction spreading between the hydrogen and hydrogen-bonded oxygen. This quantum mechanical picture fits the data far better than a purely electrostatic model.

The results indicate ~10% covalent character (the exact value is not yet published), or a mixture of idealized states. The value of 10% covalency implies a superposition state which is 90% pure electrostatic state and 10% pure covalent state. Alternatively, this can be thought of as the % contribution of the respective interactions to the overall bonding energy. Diatomic hydrogen, for example, is purely covalent (100%) with no electrostatic component, while NaCl is 75% electrostatic and 25% covalent. The covalent component of the hydrogen bond, while small in raw numerical terms, can nevertheless explain its unusual properties — just as Pauling suspected.

In some respects, the Grenoble experiment is the inverse of the typical X-ray diffraction experiment. Instead of using the wave character of light to nail down the position of particles, here the particle nature of light is used to demonstrate the wave nature of bonding electrons. Although this experiment helps to close one chapter in the history of the hydrogen bond, its fundamental significance will undoubtedly spawn other controversies. How widely does the covalency percentage vary among hydrogen bonds? How sensitive is the directionality of the bond to this percentage? Considering the importance of hydrogen-bond directionality to molecular recognition, the answer to this second question could be of use to biomolecular engineering efforts. Thus, despite these new results, the hydrogen bond will likely continue to be a subject of investigation for some time to come.

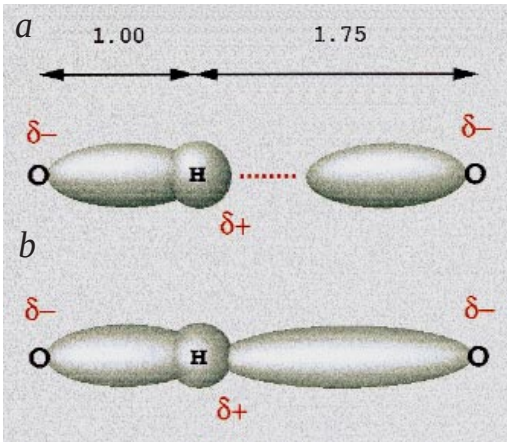

Fig. 4 Highly schematic representation of the hydrogen bond. The nearest-neighbor oxygen distance in the ice lattice is ~2.75 Å. The O-H (σ-bond) distance is ~1.0 Å, and the hydrogen-bond distance is ~1.75 Å. In ***a***, the σ-bond is depicted as the overlap of the hydrogen s-orbital and an oxygen sp^3 orbital. The hydrogen bond is depicted in terms of the polar hydrogen (δ+) for the sp^3 lone-pair (δ–) on the opposing oxygen. This is a 'classical' electrostatic picture of the hydrogen bond. In ***b***, the lone-pair electrons are shown spending a non-negligible portion of their time in the vicinity of the hydrogen, although electrostatic attraction remains the dominant effect. Although the effect may seem small, it is significant because it leads to an essentially continuous wavefunction between the two oxygens, with 'nodes' at distances of 1.0 and 1.75 Å. The described experiment[1] indicates that this picture must be used for a full understanding of the hydrogen bond.

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T.W. Martin and Zygmunt S. Derewenda are in the Department of Molecular Physiology, Health Sciences Center, University of Virginia, Charlottesville, Virginia 22908, USA. T.W.M. email: twm2n@virginia.edu and Z.S.D. email: zsd4n@virginia.edu.

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Trapped in the act of catalysis

Gideon J. Davies & Keith S. Wilson

The combination of modified substrates, freeze-trapping and site-directed mutagenesis provides an elegant description of catalysis in the ubiquitous α-amylase family.

α-Glucosidases and transferases are ubiquitous in nature where they are involved in the synthesis and breakdown of α-linked di-, oligo- and poly-saccharides. They play a crucial role in metabolic processes such as food storage and utilization and in cellular communication through modification of the glycosylation state of proteins. Inhibitors of α-glucosidases and related enzymes have therapeutic roles in the treatment of diseases such as diabetes, AIDS and cancer[1].

Cyclodextrin glycosyltransferase (CGTase) is an enzyme that converts starch and related glucans into cyclic malto-oligosaccharides, termed cyclodextrins. These compounds act as a natural sugar source for the organism but also find applied use as complexing agents in the cosmetic, food and pharmaceutical industries. CGTase is a member of the widespread α-amylase family whose members play a central role in the metabolism of starch. They have numerous industrial applications, ranging from liquifaction during the production of soluble sugars from corn starch to the formulation of detergents where they are involved in breakdown and removal of α-linked polysaccharides. The improvement of current industrial applications and the design of new oligosaccharide mimics as drugs requires a comprehensive knowledge of the catalytic mechanisms of these enzymes. Historically, many aspects of the mechanisms of the retaining α-glycoside hydrolases/transglycosylases, such as the nature of the catalytic intermediate and the role of substrate-distortion, have posed considerable technical challenges.

On page 432 of this issue of *Nature Structural Biology*, Uitdehaag and colleagues report the crystal structures of CGTase trapped in both its covalent-glycosyl-enzyme intermediate and (linear)-substrate bound forms[2]. Together, these structures illuminate the structural basis for catalysis in the α-amylase family, notably providing the first three-dimensional structure of the covalent glycosyl–enzyme intermediate of a retaining α-glycosidase/transglycosylase. This work finally resolves the controversies concerning the nature of the intermediate formed during hydrolysis and transglycosylation of α-linked sugars. The existence of a covalent intermediate and the observation of ring distortion in the substrate complex solves a historical puzzle and should aid all those embarked on the design and synthesis of α-glucosidase inhibitors as potential therapeutic agents.

Enzymatic glycoside hydrolysis

Enzymatic glycoside hydrolysis and transfer has long been a major area of study in structural biology, beginning with the pioneering work of the late David Phillips on hen egg-white lysozyme in the 1960s (for a beautiful historical perspective of this work see ref. 3). More recently, through the power of molecular biology and technical advances in X-ray crystallography, there has been an explosion of structural work in this field. There are over 70 sequence-based families of glycoside hydrolases and three-dimensional structures are now available for over 30 of these (reviewed in ref. 4). Simultaneous developments in carbohydrate chemistry have not only provided insight into the catalytic mechanism of these enzymes, but also permitted analysis of numerous

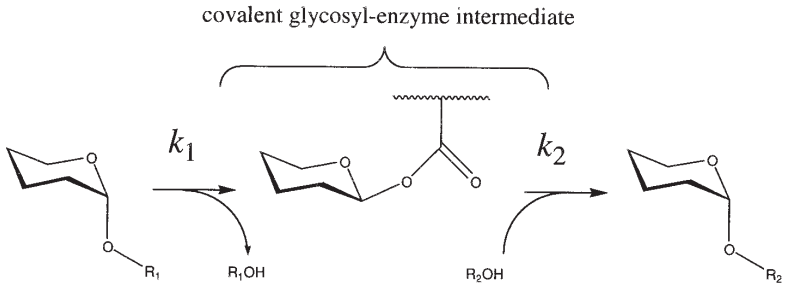

Fig. 1 The formation and breakdown of the covalent-intermediate during the retaining mechanism.