A New Look at Proton Transfer Dynamics Along the Hydrogen Bonds in Amides and Peptides

G. J. Kearley, F. Fillaux, M.-H. Baron, S. Bennington, J. Tomkinson

Vibrational spectroscopy with inelastic neutron scattering can provide spectra that are more detailed and easier to interpret than optical spectra. The spectral intensity depends on energy transfer and kinetic momentum transfer, allowing determination of the potential function. Experiments reveal that the proton involved in intermolecular hydrogen bonding in *N*-methylacetamide and polyglycine I vibrates almost independently. An ionic representation ($N^{\delta-}\cdots H^{+}\cdots O^{\delta-}$) of the hydrogen bond is more realistic than the normally accepted covalent model (NH···O). For polyglycine I, the proton experiences a local, symmetric double-minimum potential arising from dynamic exchange between the amide-like (CONH) and imidol-like (HOCN) forms of the peptide unit.

Proton transfer is of considerable importance to much of chemistry, physics, and biology (*1*). The transfer frequently occurs along an existing hydrogen bond (AH···B). Because of the rather weak interaction between a proton donor, AH, and an acceptor, B (Fig. 1), proton transfer is thought to be governed by a double minimum potential. The reaction path is characterized by the distance between the two minima (Δr), the energy difference (ΔE), and the barrier height (E_a) (*2–4*). These three parameters define the dynamics of the proton. Only a complete knowledge of these dynamics will allow us to deduce the microscopic mechanism that governs the time spent by the proton on one side of the barrier before it passes to the other. Previous studies of proton transfer have been hampered by quantum effects and strong interactions with the motions of surrounding atoms. Neutron scattering has already enabled us to measure these quantum effects directly and has shown that the effects of such interactions have been overestimated in the past.

Because the proton has low mass, quantum effects are to be expected, and the use of classical mechanics is inappropriate for the estimation of transfer rates. If the proton were a classical particle, then at very low temperatures it would always be confined to the deepest potential well. However, this is in conflict with Heisenberg's uncertainty principle: After a finite time, there is a finite probability that the proton will be in the other well. This effect is known as quantum tunneling because it occurs despite the fact that the proton has insufficient energy to jump the barrier and appears to tunnel through. Consequently, the transfer rate can be nonzero for relatively high barriers. Quantum tunneling is most dramatic when the potential has a symmetric double minimum, short Δr, and a low potential barrier. For example, with $\Delta r \approx 0.5$ Å and $E_a \approx 12$ kJ mol^{-1}, the proton residence time is $\sim 10^{-12}$ s, but it is about 100 times longer if the barrier is three times higher or if the distance between the minima is increased by 0.2 Å. Quantum tunneling disappears as ΔE increases.

Strong interactions occur if the shape of the potential energy surface is determined by the location and identity of surrounding atoms. These atoms have thermal motion, and coupling of the hydrogen atom motion to that of the heavier atoms is a key issue in the description of the proton transfer, if invoked to account for the large temperature variations of proton transfer rates. Oscillations of the A and B entities modulate the potential parameters and thus the proton transfer rate; this effect is termed "vibration-assisted tunneling" (*5*). As the A···B distance decreases, so does the distance between the two minima, the potential barrier, and the asymmetry. Tunneling then becomes more probable, and the transfer rate increases accordingly. Conversely, quantum tunneling and the transfer rate decrease as the A and B atoms separate.

The time scale of proton transfer is characterized either by the residence time in a given well or by the frequency of excursions to the other well. However, the proton also oscillates within the local potential minimum of a given well with a period between 10^{-13} and 10^{-15} s, which is much shorter than the residence time. In addition, the thermal motions of the molecules can modulate the potential over a period of 10^{-11} to 10^{-13} s. Clearly, a detailed knowledge of the proton quantum dynamics on these time scales is the prerequisite for understanding of the relationship between the microscopic structure of a system and the proton transfer rate.

The vibration-assisted tunneling mechanism has emerged from extensive studies (mostly on carboxylic acid dimers) with nuclear magnetic resonance (NMR) and quasi-elastic neutron scattering (QENS) (*6*, *7*), which probe time scales longer than about 10^{-9} s, which is considerably longer than that of proton dynamics. Consequently, the information they provide is averaged over many excursions of the proton between the two wells, and quantum effects are then not observed directly. Nevertheless, quantum effects have to be taken into account to explain deviations from the classical behavior observed with NMR and QENS. Although the resulting model provides a good fit to the average rate, it is insensitive to the elementary short time scale process. This gives potential functions that are inconsistent with vibrational spectroscopic data (*2–7*).

Vibrational Spectroscopy

Vibrational spectroscopy is the only technique that measures atomic oscillations on a time scale comparable with the proton dynamics (10^{-12} to 10^{-15} s). These techniques are used to measure the frequency of the proton oscillation within the wells (stretching frequency). In the case of carboxylic acid dimers, these frequencies are too high to be consistent with the rather low potential barriers estimated from NMR and QENS studies. Unfortunately, optical spectroscopies (infrared and Raman) have

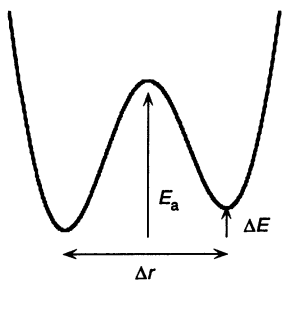

Fig. 1. Schematic illustration of the double minimum potential governing proton transfer along a hydrogen bond AH···B.

G. J. Kearley is at Institut Laue Langevin, BP 156, 38042 Grenoble Cedex 9, France. F. Fillaux and M.-H. Baron are at the Laboratoire de Spectrochimie Infrarouge et Raman, 2 Rue Henry Dunant, 94320 Thiais, France. S. Bennington and J. Tomkinson are at Rutherford Appleton Laboratory, Chilton, OX11 0QX, United Kingdom.

disadvantages for the study of proton transfer that preclude a complete characterization of the potential. With hindsight, it is not surprising that characterization of proton-transfer mechanisms with optical techniques has been the source of some long-standing controversies. For proton-transfer studies, the main difficulties arise from the nonspecific sensitivity for proton vibrations and the lack of a rigorous theoretical framework for the interpretation of observed intensities.

Most of the difficulties encountered in the analysis of the infrared and Raman spectra are overcome with inelastic neutron scattering (INS) spectroscopy. Here neutrons, instead of photons, are used to excite vibrations. The same transitions as in the optical techniques are observed but with completely different intensities because the neutron scattering process is entirely attributable to nuclear interactions. Each atom is characterized by its nuclear cross section, which is independent of its chemical bonding. The intensity for any transition is simply related to the atomic displacements scaled by scattering cross sections. The technique is particularly well adapted for proton transfer because the cross section of the proton is almost 10 times greater than that for any other atom. This proton selectivity can be further exploited because the deuterium atom (^{2}H) has a very much smaller cross section than the proton. For a system with several protons, specific deuteration of some sites therefore simplifies the observed signals. As a result, INS intensities provide information on the proton dynamics that can be analyzed with greater confidence than the corresponding infrared and Raman spectra.

The shape of the potential function has been determined accurately for ionic systems containing strong hydrogen bonds (*8*). The analysis of the INS intensities has revealed that the proton dynamics are almost completely independent of the dynamics of the surrounding atoms and that the tunneling bands are intense and nar-

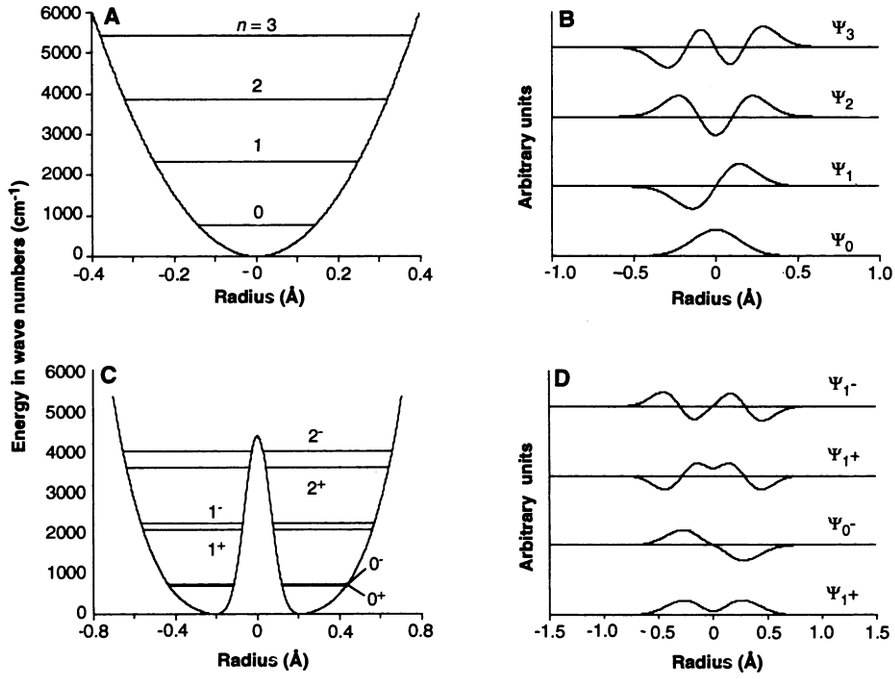

Fig. 3. (**A**) Energy levels and (**B**) wave functions for a harmonic potential function and (**C**) energy levels and (**D**) wave functions for a double minimum potential function. The double minimum potential determines the proton dynamics along the hydrogen bond in polyglycine I.

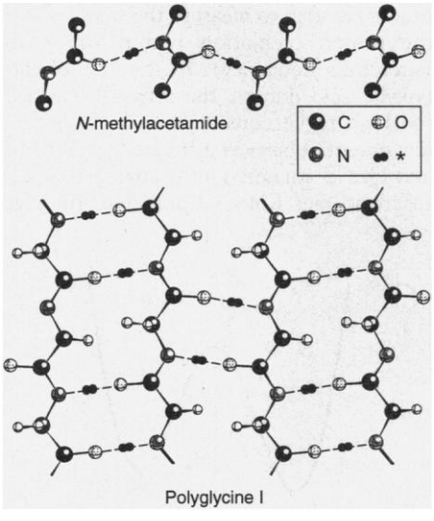

Fig. 2. Illustration of the hydrogen bonding arrangement in the structures of the *N*-methylacetamide (*16*) and polyglycine I (*17*). The small filled circles (*) represent the two sites among which the hydrogen bonding protons are delocalized.

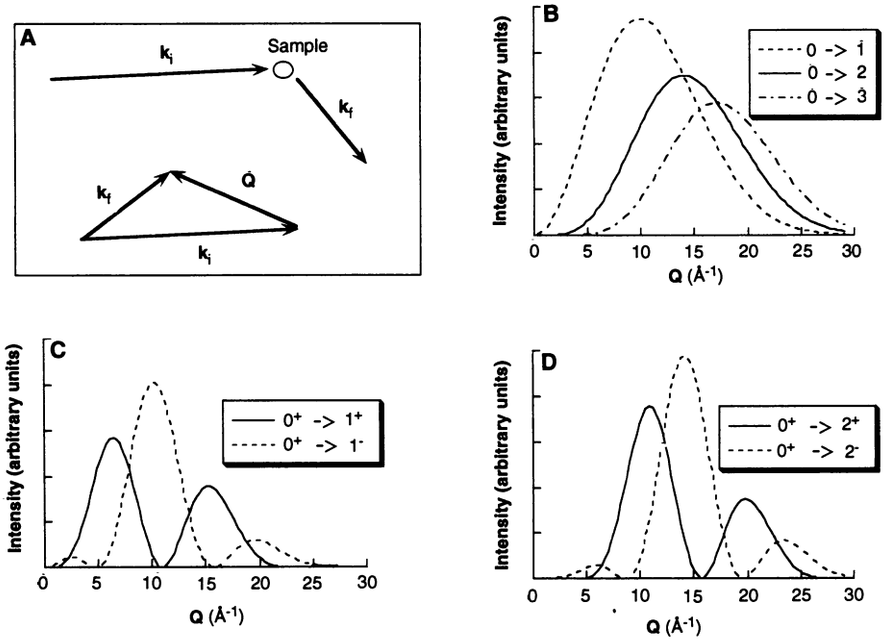

Fig. 4. (**A**) Definition of the kinetic momentum transfer, **Q**, in a scattering experiment. The wave vectors for the incident and scattered plane wave, $\mathbf{k}_i$ and $\mathbf{k}_f$, respectively, are defined as $2\pi/\lambda$, where λ is the wavelength. (**B**) The **Q**-dependencies of the INS intensities for transitions between vibrational states for a harmonic potential. (**C** and **D**) The **Q**-dependencies of the INS intensities for transitions between vibrational states for a double minimum potential governing the proton dynamics along the hydrogen bond in polyglycine I.

row. The potential barriers are much higher than those previously proposed for carboxylic acids with weaker hydrogen bonds (*2–7*). It appears that the vibration-assisted tunneling model is not relevant in systems where the proton transfer dynamics are dominated by quantum effects.

Proton Transfer in Amides and Polypeptides

One of the recent highlights of INS in vibrational spectroscopy has been the study of *N*-methylacetamide ($CH_3CONHCH_3$) (Fig. 2) and polyglycine $(-CO-CH_2-NH-)_n$. In the solid state, the polyglycine adopts two secondary structures: the antiparallel chain rippled-sheet form (I) (Fig. 2) and the triple helix, collagen-like form (II). These molecules are simple models for the peptide unit, which is of central importance to many biological structures and processes. The heavy atom skeleton of this unit (C, O, and N) is almost planar, with the C=O and N–H bonds trans to each other across the central C–N bond. Resonance between the two mesomeric forms X and Y was proposed by Pauling (*9*) with form X being dominant

$$O{=}C{-}N{-}H\ (X) \leftrightarrow O^{-}{-}C{=}N{-}H^{+}\ (Y)$$

In the crystalline state, the N–H group of one molecule links to the C=O group of a neighbor to create a hydrogen-bonded NH···O bridge, with N···O ≈ 2.8 Å, which is similar to those observed for proteins.

The INS spectra of the partially deuterated analogs $CD_3CONHCD_3$ (*10*) and $(-CO-CD_2-NH-)_n$ (*11*) have been studied to investigate the proton dynamics in the hydrogen bonds. Surprisingly, most of the conclusions previously obtained with INS for hydrogen-bonded dimers in ionic crystals also apply to these systems, although they are molecular in nature: In all cases, the proton dynamics are isolated from the dynamics of the molecular backbone. In addition, the use of INS intensities reveals that the previous estimate for the vibrational frequency of the proton, based on the optical techniques, is almost a factor of 2 too high. This result is obviously in contrast to the normally accepted view and implies a significant increase of the N–H bond length. Consequently, the covalent bond between the N and H atoms is actually considerably weaker than was thought previously, and instead of the covalent representation of the hydrogen bond (NH···O), the ionic model ($N^{\delta-}\cdots H^{+}\cdots O^{\delta-}$) appears to be more realistic. This weaker N–H bond is a strong incentive to reexamine the proton dynamics thoroughly in order to estimate the degree of proton transfer from the N to the O atom.

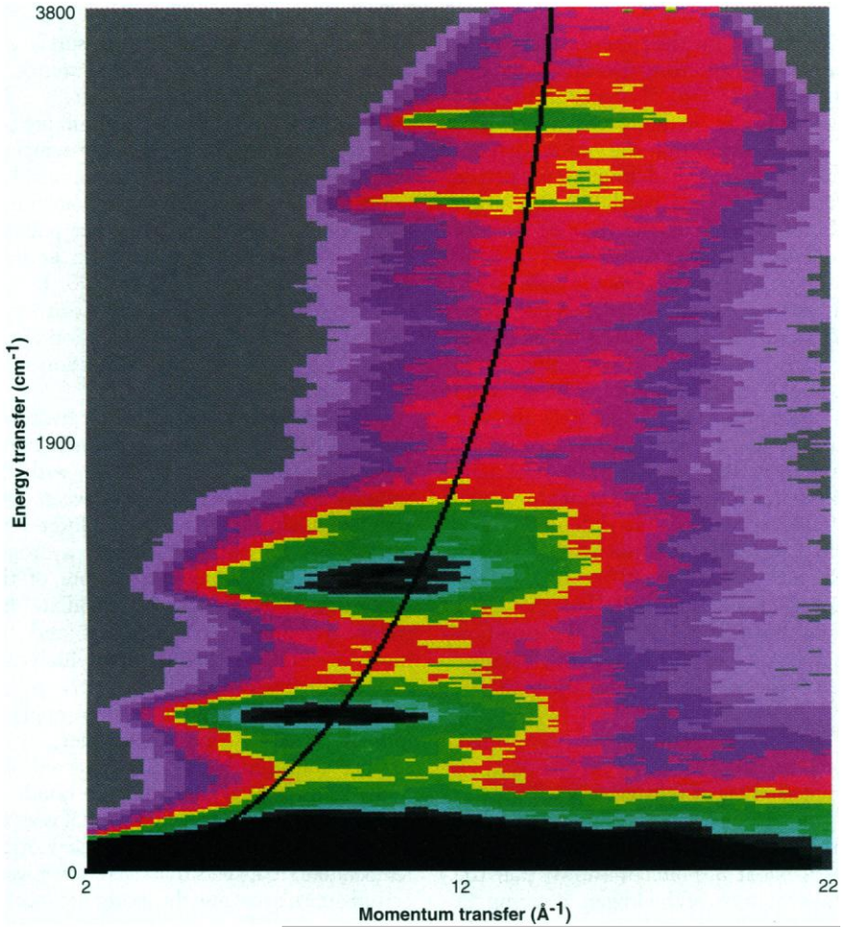

Fig. 5. Map of $S(\mathbf{Q},\omega)$ for polyglycine I at 20 K. The black regions denote maximum intensity; the line corresponds to the maximum intensity for fully localized protonic modes.

Single and Double Minimum Potential Functions

Quantum mechanics applied to a proton in a harmonic (single minimum) potential [for example, $V(x) = \frac{1}{2}Fx^2$, where F is the force constant and x is the displacement] gives vibrational states characterized by a quantum number $n = 0, 1, 2, \ldots$, wave functions Ψ_n, and energy levels $E_n = (n + 1/2)\hbar\omega_o$, where $\hbar$ is Planck's constant divided by 2π and ω_o is the vibrational frequency (Fig. 3, A and B). At low temperature, only the ground state is populated; its energy, $E_o = \hbar\omega_o/2$, is not zero, and the probability distribution for the proton position, given by $|\Psi_o|^2$, has a finite width. In principle, at low temperatures optical techniques can only observe the $0 \rightarrow 1$ transitions, whereas any transition can be observed with INS.

In a symmetric double minimum potential, the effects specific to quantum behavior of the proton appear (Fig. 3, C and D). Each vibrational level is split into two sublevels, which are either symmetric (Ψ^+) or antisymmetric (Ψ^-). Even in the ground state, far below the top of the potential barrier, the proton is not confined to a particular well. The ground state splitting ($0^+ \leftrightarrow 0^-$) is termed tunnel splitting, and the corresponding frequency can be thought of as the inverse of the time taken for the proton to pass between the two wells. The interaction of photons with matter gives symmetry-related selection rules: Only transitions between levels of different symmetry can be observed in the infrared, whereas only transitions between levels of the same symmetry can be observed in Raman. All transitions are observable in the INS spectrum.

Optical spectra support the presence of a symmetric double minimum potential along the hydrogen bond in polyglycine, but because a multitude of effects, arising from changes in electron density, control the intensities in these spectroscopies, it is impossible to characterize the double minimum unambiguously.

Variation of Vibrational Spectrum with Momentum Transfer

Fortunately, recent advances in INS spectroscopy shed new light on the double minimum problem, particularly as we are

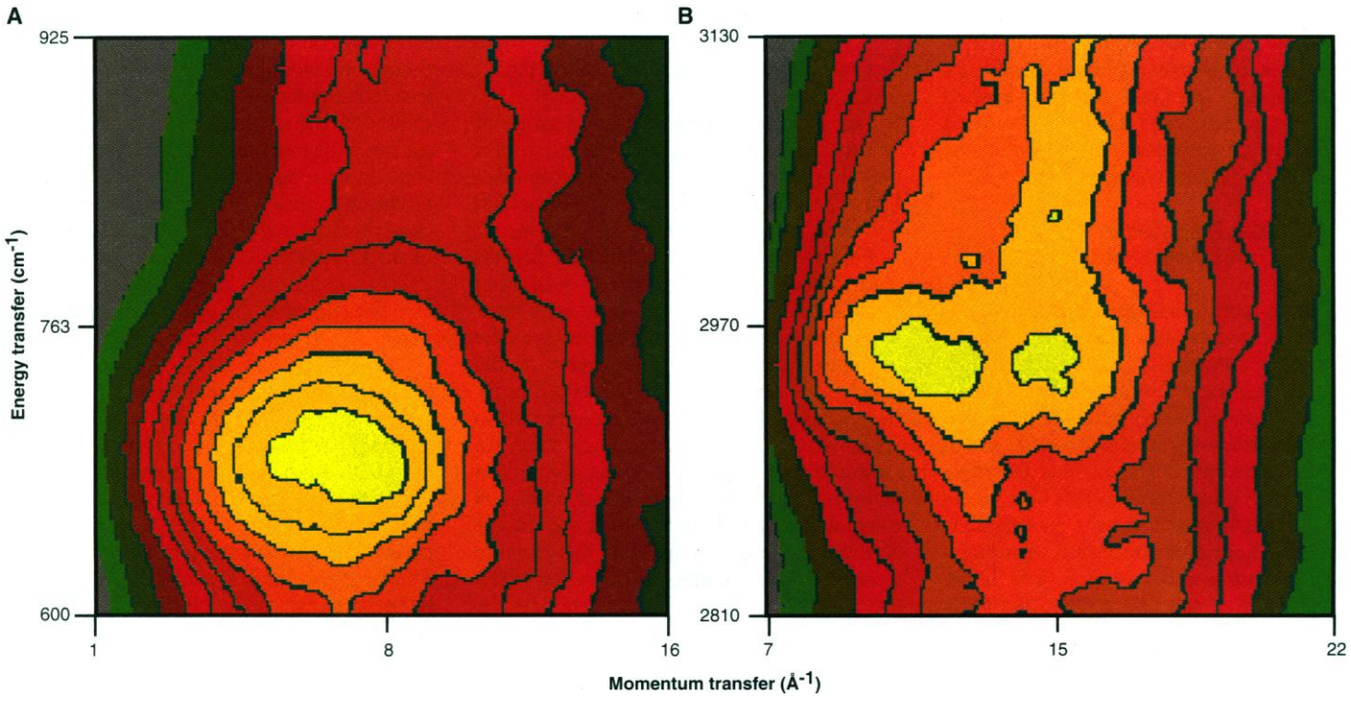

Fig. 6. Detailed view of the $S(\mathbf{Q},\omega)$ map for polyglycine I at 20 K for (**A**) the hydrogen bonding proton bending mode perpendicular to the mean plane of the peptide unit and (**B**) the hydrogen bonding proton stretching mode. The yellow regions correspond to maximum intensity.

able to measure spectra over a range of kinetic momentum transfer ($\mathbf{Q}$). This is the vector difference between the incident and the scattered wave vectors (Fig. 4A); for optical spectra $\mathbf{Q} \approx 0$. The variation of the INS spectral intensity with the angle at which the scattered neutron is measured contains spatial information of the proton wave functions for this vibrations. Some INS spectrometers allow the momentum transfer, $\mathbf{Q}$, and the energy transfer, ω, to be varied independently and yield the full scattering functions, $S(\mathbf{Q},\omega)$. We can then use the $\mathbf{Q}$ dependence of the intensity to obtain the shapes of the potential functions for the various vibrations (Fig. 4, B through D). For a harmonic oscillator, the value of $\mathbf{Q}$ at which the maximum intensity occurs gives a direct estimate of the effective oscillator mass. This is related to the eigenvector that describes the motions of the different atoms (in the present case, just the proton). The $\mathbf{Q}$ dependence also provides a straightforward distinction between single and double minimum potentials (Fig. 4, B through D).

When we examine the map of the scattering function, $S(\mathbf{Q},\omega)$, for the partially deuterated polyglycine I (Fig. 5), we see the expected "islands" of intensity arising from protonic modes. Normally, in a molecule where hydrogen is bound to heavier atoms, the different reduced masses cause the disposition of these islands to be somewhat scattered. This scatter normally reflects the different oscillator masses, which are a result of the varying involvement of the heavier atoms in the proton vibrations. However, the intensity maxima follow a parabolic curve (Fig. 5), with each island corresponding to oscillations of a bare proton. This confirms that the proton dynamics really are separated from those of the backbone. The same parabolic disposition of islands is seen in the $S(\mathbf{Q},\omega)$ map of *N*-methylacetamide.

A straightforward harmonic case is exemplified by the out-of-plane bending mode (at 765 cm^{-1}), which corresponds to displacements of the proton perpendicular to the plane of the peptide unit. The observed map compares very favorably with that calculated for a bare proton undergoing harmonic oscillation (Fig. 6A). The map for the symmetric double minimum potential function is markedly different and is clearly compatible with the $S(\mathbf{Q},\omega)$ map of the higher transitions ($0^+ \rightarrow 2^+$) of the stretching mode (Fig. 6B). For the lower energy transition ($0^+ \rightarrow 1^+$), the situation is complicated by overlap of the bending mode parallel to the peptide unit plane.

Conclusion

The full analysis of INS vibrational spectra with the use of spectral intensities and momentum transfer dependence is in its infancy, but it has already shown that the previous picture of hydrogen bonding in peptides is seriously in error. Double minimum potentials have been postulated previously, but the ability of INS to measure the wave functions directly is surely the most solid evidence for the existence of double minima to date. In fact, for *N*-methylacetamide, the double minimum potential is symmetric only at room temperature but becomes asymmetric at very low temperature as proton transfer from the N atom to the O atom occurs. For polyglycine, the potential is symmetric at least within the range of 20 to 300 K. At present, vibrational spectroscopy cannot be used to determine the exact location of the double minimum; for this, diffraction measurements are necessary.

In the picture that emerges for hydrogen bonding in polyglycine I, hydrogen bonds form a two-dimensional network, with the protons being delocalized between two equivalent sites (Fig. 3). The inter- and intrachain coupling between hydrogen bonds is negligible. The structure of the peptide unit is strictly intermediate between the amide-like (CONH) and the imidol-like (HOCN) structures, which cannot be distinguished. Preliminary results suggest that the same conclusions apply to form II. Therefore, proton transfer, an essential process in biology, is achieved naturally as a result of the hydrogen bond.

Because the double well potential is asymmetric in *N*-methylacetamide below room temperature, it appears that even rather weak perturbations can cause the protons to localize in one or the other potential wells, favoring either the amide-like or imidol-like entities. At the present stage, the ability of solid-state

NMR to follow proton transfer is not clear. Such studies are rather scarce (*12–15*), and none of them have been conclusive for the proton transfer dynamics.

The symmetric double minimum potential for the asymmetric hydrogen bond between peptide units shows that such potentials can occur even for hydrogen bonds that are not formally symmetric (A···H···A). However, this picture has been derived within the Born-Oppenheimer approximation applied at two distinct levels: (i) the electrons follow the atomic nuclei adiabatically, and (ii) the light protons follow the heavy atoms adiabatically. Use of INS reveals that this framework is not adequate to describe the dynamics in hydrogen bonds.

The impact of INS spectroscopy is bound to extend far beyond the study of proton transfer along hydrogen bonds. Many fields of research concerned with proton mobility in the solid state will benefit from recent and future developments of INS techniques.

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RESEARCH ARTICLE

Antibody Catalyzed Cationic Cyclization

Tingyu Li, Kim D. Janda,* Jon A. Ashley, Richard A. Lerner*

Two major goals for the design of new catalysts are the facilitation of chemical transformations and control of product outcome. An antibody has been induced that efficiently catalyzes a cationic cyclization in which an acyclic olefinic sulfonate ester substrate is converted almost exclusively (98 percent) to a cyclic alcohol. The key to the catalysis of the reaction and the restriction of the product complexity is the use of antibody binding energy to rigidly enforce a concerted mechanism in accord with the design of the hapten. Thus, the ability to direct binding energy allows the experimenter to dictate a reaction mechanism which is an otherwise difficult task in chemistry. New catalysts for cationic cyclization may be of general use in the formation of carbon-carbon and carbon-heteroatom bonds leading to multiring molecules including steroids and heterocyclic compounds.

The study of carbocations has added to the understanding of reaction mechanisms in organic chemistry (Fig. 1) (*1*). Nevertheless, controlling the reaction pathways of this highly reactive species is not easy. Among the many transformations where carbocations appear on the reaction pathway, cationic cyclization is one of the most important carbon-carbon bond forming processes in chemistry and biochemistry (*2–17*). Cationic processes are central to many synthetic strategies as well as the polyene cyclization cascade that leads to the formation of steroids. Some of the earliest synthetic work on cationic cyclization reactions was that of Johnson and his colleagues, who studied the formation of six-membered rings from acyclic unsaturated compounds (*2*). Their work led to the recognition that the process must be initiated in a way that generates a cationic center on carbon without affecting the olefinic bonds.

Typically, a cationic cyclization reaction is initiated by the formation of a carbocation, either by electrophilic addition to a double bond or by ionization at a sp^3 hybridized carbon. The reaction is thought to proceed via a transition state in which the reactants adopt a quasi-chairlike conformation, thereby allowing participation of the olefinic bond in what is essentially a concerted transformation.

To catalyze cationic cyclization requires control of the initial generation and stabilization of the carbocation as well as the attendant entropic and stereoelectronic parameters intrinsic to the cyclization reaction. Antibodies should, in principle, be ideal catalysts for initiation and control of this process in that they have been shown to be capable of catalyzing complex reactions in which it was necessary to simultaneously neutralize point charges, overcome entropic barriers, and provide a chiral binding pocket for stereoselectivity (*18*). In essence, the problem reduces to that of generation of the carbocation in an environment that fosters and controls the cyclization reaction. We now describe the implementation of these concepts to achieve antibody catalyzed cationic cyclization.

We studied the classical system of Johnson in which the initiating carbocation is formed by the solvolysis of sulfonate esters; we used **1** as a substrate (Fig. 2) (*2*). At acidic pH several zwitterionic or cationic species or both (Fig. 2) were developed in the transition state, of which **2** is representative. Although such systems can undergo solvolysis-cyclization reactions with electron-rich olefins, they are not always useful because the yield is poor and complex mixtures of products are produced (*19*). However, antibody catalysis permits selective control of the mechanism of the solvolysis of **1**, thereby reducing the complexity of the reaction and improving the yield of desired products.

The reaction and hapten design. To catalyze the cationic cyclization of **1**, we designed a hapten that induced an antibody that simultaneously facilitates the cleavage of the sulfonate and controls the conformation of the substrate in the transition state such that the olefin is properly aligned to participate in the reaction. The cyclic *N*-oxide **3** would seem to be an ideal hapten to induce antibodies capable of catalyzing release of the sulfonate from **1**. The anionic oxygen should elicit a functionality in the antibody capable of operating by way of a process that we have termed "bait and switch" catalysis to stabilize the developing negative charge on the departing sulfonate

The authors are at the Scripps Research Institute, Departments of Molecular Biology and Chemistry, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA.

*To whom correspondence should be addressed.