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Trans-Hydrogen-Bond $^{h2}J_{NN}$ and $^{h1}J_{NH}$ Couplings in the DNA A−T Base Pair: Natural Bond Orbital Analysis

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Received September 26, 2001

The recent recognition that trans-hydrogen-bond (trans-H-bond) NMR J couplings in biomolecules can be resolved and interpreted in structural terms[1−8] offers an unambiguous way of detecting and analyzing H-bonds in biomolecules. However, measurements of trans-H-bond couplings in NH···N H-bonds, for example, $N_1 \cdots H_3-N_3$ in the DNA A−T base pair (Figure 1), provided the counterintuitive finding that the *two-bond* coupling ($^{h2}J_{NN} \approx 9$ Hz)[4] is larger than the *one-bond* coupling ($^{h1}J_{NH} \approx 3$ Hz).[5,8] Natural bond orbital (NBO) analysis described here demonstrates that $^{h2}J_{NN}$ and $^{h1}J_{NH}$, are determined largely by three terms: two Lewis-type contributions (the single-orbital contribution from the adenine lone pair and the contribution from the σ_{N3H3} natural bond orbital of the thymine ring) and one contribution from pairwise delocalization of spin density (between the lone pair in adenine and the σ^* antibonding orbital linking N_3 and H_3 of thymine). For $^{h2}J_{NN}$ coupling, all three contributions are positive; whereas for $^{h1}J_{NH}$ coupling, the delocalization term is negative, and the other two terms are positive, resulting in a small net positive coupling constant. This result rationalizes the experimental findings and demonstrates that the same hyperconjugative and steric mechanisms that stabilize the H-bond are involved in the transmission of J coupling information. The $N_1 \ldots H_3-N_3$ H-bond of the DNA A−T base pair is found to exhibit significant covalent character, but steric effects contribute almost equally to the trans H-bond coupling.

Unlike other properties of NMR that can be understood in terms of pseudo-classical physics, J coupling is purely a quantum mechanical phenomenon. Trans-H-bond J couplings, which speak directly to the nature of the hydrogen bond, have motivated a number of computational studies.[5,9−13] The discovery of trans-H-bond J coupling has lead some to conclude that H-bonds exhibit covalent character[1,9,14] (in accord with previous theoretical suggestions),[15] while others have stressed that covalency is not a requirement for trans-H-bond coupling.[13] The goals of the present model study of the DNA A−T base pair were: (1) to determine, by directly computing NBO contributions to couplings, the relative importance of covalent and nonbonding interactions to the transmission of coupling through H-bonds, and (2) to delineate the major contribution to the observed J coupling in terms of localized energetics responsible for H-bonding so as to determine what relationship exists between J coupling and the covalency of the H-bond.

In contrast to previous studies in which J coupling was related indirectly to computed valence-bond orders,[9] and charge densities,[13] the present analysis utilized the concepts and methods of NBO theory. Briefly, the NBO package includes a suite of methods for describing the N-electron wave function $\psi(1,2,...,N)$ in terms of localized orbitals or configurations that are closely tied to chemical bonding concepts.[15,16] Underlying these methods are sets of localized intrinsic "natural" atomic orbitals (NAO), natural bond orbitals (NBO), and semi-localized molecular orbitals (NLMO) that correspond closely to the Lewis structure representations used by chemists. To calculate the Fermi-contact contributions to J couplings, the single finite perturbation method of Pople et al.,[17,18] which has been reintroduced recently by Barfield and co-workers,[19] was used to produce wave functions with J coupling information "built-in", which were then analyzed in the framework of natural J coupling (NJC)[20] as implemented in the NBO 5.0 software package.[21] The resulting set of NBOs was used to decompose the calculated J coupling constants into their constituent contributions.[20] The resulting coupling contributions (Table 1) could then be related to the energetic features of the H-bond to determine whether the J coupling is associated with covalent or noncovalent features of the bond. The net calculated J couplings, 9.31 Hz for $^{h2}J_{NN}$ and 2.64 Hz for $^{h1}J_{NH}$, are in good agreement with the expected experimental values.[7] Table 1 also displays the leading contributions to the $^{h2}J_{NN}$ and $^{h1}J_{NH}$ couplings:[20] $J^{(L)}{}_{LP(N1)}$, the single NBO Lewis contribution from the adenine N_1 lone pair (LP_{N1}); $J^{(L)}{}_{\sigma N3H3}$, the contribution from the Lewis σ_{N3H3} NBO of the thymine; and $J^{(deloc)}{}_{LP(N1)\to\sigma^*(N3H3)}$, the pairwise delocalization of spin density between the adenine N_1 lone pair (LP_{N1}) NBO and the thymine σ^*_{N3H3} NBO.

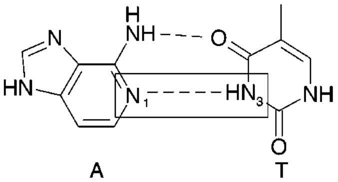

Figure 1. DNA A−T base pair model. The box highlights the hydrogen bond between N_1 of adenine (A) and N_3 of thymine (T).

The total contribution from the natural Lewis structure, $J^{(L)}$, to the $^{h2}J_{NN}$ coupling (4.16 Hz) is smaller than the corresponding contribution to the $^{h1}J_{NH}$ coupling (6.71 Hz). The total contributions from delocalization, $J^{(deloc)}$, are nearly equal and opposite for the two couplings (4.85 Hz for $^{h2}J_{NN}$ and −4.54 Hz for $^{h1}J_{NH}$). The contribution from repolarization of the NBOs, $J^{(repol)}$, is small.

From Table 1, it is apparent that contributions from the delocalization of spin density play a significant, if not dominant, role in determining the sign and magnitude of the trans-H-bond J couplings. The importance of hyperconjugative delocalization in determining the $^{h2}J_{NN}$ and $^{h1}J_{NH}$ couplings indicates appreciable electron-sharing across the hydrogen bond. Therefore, it can be concluded that the covalent character of the $N_1 \cdots H_3-N_3$ hydrogen

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10.1021/ja017169c CCC: $22.00

Table 1. Leading Calculated Contributions[20] to the $^{h2}J_{NN}$ and $^{h1}J_{NH}$ Couplings for the A−T Base Pair[a]

	$J^{(L)}_{LP(N1)}$	$J^{(L)}_{\sigma N3H3}$	$J^{(deloc)}_{LP(N1)\rightarrow\sigma^*(N3H3)}$	$J^{(L)}$	$J^{(deloc)}$	$J^{(repol)}$	net
$^{h2}J_{NN}$ (/Hz)	2.04	3.08	3.68	4.16	4.85	0.30	9.31
$^{h1}J_{NH}$ (/Hz)	1.29	6.33	−5.80	6.71	−4.54	0.48	2.64

[a] Calculations on the model (Figure 1) were performed with the B3LYP[23] hybrid density functional using the 6-311G** basis set as implemented in Gaussian 98.[24] The three terms on the right are the full calculated values for the Lewis, delocalization, and repolarization contributions, which sum to the net calculated coupling.

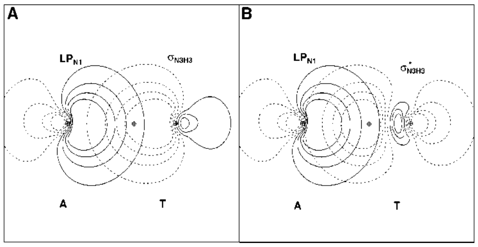

Figure 2. Pre-natural bond orbital contours: (A) overlap between the N_1 lone pair in adenine (LP_{N1}) and the σ-bonding orbital between N_3 and H_3 of thymine (σ_{N3H3}); (B) overlap between the N_1 lone pair in adenine (LP_{N1}) and the σ^* antibonding orbital between N_3 and H_3 of thymine (σ^*_{N3H3}).

bond in the A−T base pair plays a key role in determining the $^{h2}J_{NN}$ and $^{h1}J_{NH}$ coupling constants.

Previous work has demonstrated that the Lewis NBO contributions to J couplings correlate with their corresponding unfavorable steric exchange energies,[20] which can be estimated from natural steric analysis (NSA).[22] Pre-NBOs (PNBOs), which are a set of localized Lewis NBO precursors that lack the final interatomic orthogonalization step, provide a convenient way to visualize interactions between orbitals in different bond regions, because their overlap is proportional to their interaction energy.[20] For the A−T base pair, the steric interactions between the σ_{N3H3} and the LP_{N1} PNBOs (Figure 2A), which are associated with the leading Lewis contributions to J coupling ($J^{(L)}_{\sigma(N3H3)}$ and $J^{(L)}_{LP(N1)}$; see Table 1) give a computed energy of 27.19 kcal/mol. The existence of Lewis coupling contributions of such a large magnitude mediated by noncovalent Pauli repulsion between the σ_{N3H3} and the LP_{N1} NBOs supports previous ideas that covalent bonds are not necessary for the transmission of J coupling across a hydrogen bond.[13]

In analogy to the steric-exchange energy, pairwise NBO delocalization contributions ($J^{(deloc)}$) correlate with donor−acceptor delocalization energies, which can be calculated from second-order perturbation theory.[20] For the A−T base pair model, the NBO interaction associated with the $J^{(deloc)}_{LP(N1)\rightarrow\sigma^*(N3H3)}$ can be regarded as the second-order hyperconjugative delocalization energy arising from the donation of electron (spin) density from LP_{N1} into σ^*_{N3H3} ($LP_{N1}\rightarrow\sigma^*_{N3H3}$). Figure 2B displays a two-dimensional contour plot of the donor−acceptor interaction between the LP_{N1} and σ^*_{N3H3} PNBOs. This interaction energy is computed to be −35.16 kcal/mol; this outweighs the steric energy, providing a net stabilization of 7.97 kcal/mol. Although a number of additional, smaller interactions contribute to the strength of the H-bond, this example illustrates how the H-bond can be visualized as a delicate balance between favorable hyperconjugative interactions and unfavorable steric interactions. Both of these interactions are intrinsically associated with the exchange region [i.e., the short-range region of "chemical" or "valence" forces, dominated by quantum-mechanical orbital interactions of filled−unfilled (hyperconjugative) or filled−filled (steric) type], and reflect their essential *quantal* nature. Both mechanisms can play an important role in trans-H-bond coupling.

Acknowledgment. Supported by NIH Grant RR02301 to J.L.M and a traineeship to S.J.W. from NIH Molecular Biophysics Training Grant GM08293. Software development and computations utilized a four-processor server graciously donated by the Microsoft Corporation. We thank Professor David Case (Scripps Research Institute) for the A−T base pair structural model and helpful discussions.

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JA017169C