First Evidence of Anchimeric Spin Delocalization in Tryptophan Radical Cation[1]

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Neutral and cationic amino acid radicals,[2,3] including glycyl, tyrosyl, cysteinyl, and tryptophanyl,[4−6] are implicated in DNA and protein damage, radical scavenging, and enzymatic reactions such as electron transfer. Although long-range electron transfer (ET) along specific paths in proteins is controversial and the explicit roles of amino acid side chains, peptide bonds, and hydrogen bonds are uncertain,[7,8] tryptophan radical cations ($TrpH^{\bullet+}$) are suspected as intermediate electron acceptors in DNA photolyase[4] (DNP) and cytochrome *c* peroxidase[5] (CcP). This contribution describes hybrid Hartree−Fock/density functional[9] (HF/DF) calculations for $TrpH^{\bullet+}$ implying a conformationally dependent, *through-space* spin delocalization from the π system of the indole side chain onto the alanyl chain. Thus, we observe an intramolecular interaction between peptide Lewis bases and the singly occupied π orbital of the cationic indole ring.[10] For simplicity, we term this unprecedented effect anchimeric spin delocalization (ASD) by analogy with the anchimeric effect in organic chemistry—the neighboring group stabilization of carbocations—and discuss the potential implications of ASD for long-range ET (LRET).

Table 1. Spin Density Distributions Calculated for **1** and **2** and Their Respective Diamides, Indolyl Radical Cation,[a] and Indolyl Radical Cation with Formamides Modeling CcP-I[b]

spin densities[e]	**1**	**2**	**1**-diamide[c]	**2**−diamide	$IndH^{\bullet+}$ + $2NH_2CHO$[d]	$IndH^{\bullet+}$
N1	0.09	0.12	0.09	0.12	0.09	0.11
C2	0.18	0.18	0.18	0.17	0.18	0.17
C3	0.34	0.28	0.35	0.27	0.36	0.34
C4	0.23	0.23	0.23	0.23	0.27	0.30
C5	−0.09	−0.06	−0.09	−0.07	−0.10	−0.11
C6	0.18	0.14	0.19	0.15	0.20	0.23
C7	0.03	0.09	0.02	0.09	0.05	0.08
C8	0.05	0.006	0.05	0.01	0.05	0.03
C9	−0.10	−0.09	−0.11	−0.09	−0.10	−0.09
C_β	−0.02	−0.01	−0.02	−0.009		
C_α	0.03	0.02	0.03	0.03		
N_α	0.05	0.08	0.03	0.06		
C′	<0.001	0.01	−0.005	0.008		
O_α	0.04	0.007	0.05	0.03		
N_β (OH)	0.01	0.001	−00.01	0.005		
$O_{\alpha'}$			0.01	0.03		
$C_{\alpha'}$			−0.005	−0.01		
$O^{His-175}$ *f*					0.04	
$O^{Met-230}$					0.02	
total alanyl chain	0.11	0.11	0.08	0.14		

[a] Walden, S. E.; Wheeler, R. A. *J. Chem. Soc., Perkin Trans. 2* **1966**, 2663−2672. Walden, S. E.; Wheeler, R. A. *J. Phys. Chem.* **1996**, *100*, 1530−1535. [b] The relative magnitudes of the predicted spin densities at C3 (Cγ) and C2 (Cδ1) are consistent with photochemically induced nuclear polarization experiments (Stob, S.; Kaptein, R. *Photochem. Photobiol.* **1989**, *49*, 565−577; Hore, P. J.; Broadhurst, R. W. *Prog. NMR Spectrosc.* **1993**, *25*, 345−402) but not with the electron nuclear double-resonance experiments for CcP.[14] [c] These numbers are from a partial optimization using the trcconf1 optimized geometry but optimizing internal coordinates in the amino acid backbone. [d] This is from a single-point calculation based on geometries obtained from $IndH^{\bullet+}$ + $1NH_2CHO$ calculations modeling each interaction separately. [e] Experimental spin densities for $TrpH^{\bullet+}$ from CcP compound I[14] N1 = 0.14, C2 = 0.35, C3 = 0.41, and C5 = −0.07. [f] $O^{His-175}$ and $O^{Met-230}$ indicate the formamide oxygen atoms placed in positions with respect to the indole ring similar to the carbonyl oxygens of the respective residues in the CcP compound I Laue structure.[17]

Of the five conformations examined,[11] two exhibit spin delocalization onto the heteroatoms of the side chain. These two conformations (**1** and **2**; cf. **3** for atom numbering[12]) are shown in Newman projections along the $C^\beta C^3$ ($C^\beta C^\gamma$) bond. The distinguishing feature of **1** and **2** is that the alanyl chain is oriented so that either the nitrogen or the carbonyl oxygen is as close as 2.790 Å to a carbon on the ring, that is, significantly closer than the sum of the van der Waals radii.[13] We believe this close contact results in direct polarization and spin delocalization onto the heteroatom. In *vivo* this could also result in a stabilization of the charge on the indolic ring system. The amount of delocalized spin on each atom is small but significant

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(9) The hybrid Hartree−Fock/density functional method B3LYP with the 6-31G(d) basis set was used for this work through the program suite GAUSSIAN94 with default options for geometry optimizations.[9a,b] An explanation of the method and its applicability to this question are addressed in Supporting Information. (a) Foresman, J. B.; Frisch, Æ. *Exploring Chemistry with Electronic Structure Methods*; Gaussian: Pittsburgh, 1996. (b) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *GAUSSIAN94*; Gaussian, Inc.: Pittsburgh, 1995.

(10) We have also analyzed several conformations of tryptophanyl neutral radical but find no unpaired spin density on the alanyl chain.

(11) Conformational analysis using the MOPAC 6.0 program[11a] with the AM1 Hamiltonian[11b] approximation yielded 18 distinct conformations as stable points on the potential energy surface. The details of the conformational analysis are submitted as Supporting Information. In general, full optimizations were conducted from stepwise starting geometries obtained by varying the torsions around three bonds, $C^\alpha C'$, $C^\alpha C^\beta$, and $C^\beta C^\gamma$. Of these 18 conformations, 4 of the 5 lowest in energy (trcconf1−4) were selected for full optimizations at the B3LYP/6-31G(d) level. An additional B3LYP/6-31G(d) optimization was performed beginning with a structure (trcconfsp) modified from the x-ray crystal structure of L-tryptophan hydrochloride[11c] by removal of the HCl. Supporting information also includes a comparison (Table 1, Supporting Information) of alanyl chain dihedral angles for these 5 conformations, the Laue structure of Trp-191 from CcP-I, and the X-ray structure of Trp-306 of DNP. (a) Stewart, J. J. P. *QCPE Bull.* **1983**, 455. (b) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909. (c) Takigawa, T.; Ashida, T.; Sasada, Y.; Kakudo, M. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 2369−2378.

(12) The Greek letter designations shown in **3** derive from the IUPAC recommendations for biochemical nomenclature[12a] as do the dihedral angles given in Table 1 (Supporting Information). The numbers in parentheses for the indolic ring atoms derive from standard numbering for the indole ring and are used at times for simplicity. Hoffmann-Ostenhof, O.; et al. *Biochemistry* **1970**, *9*, 3471−3479.

(13) Table 2 in the Supporting Information includes the distances of the Lewis bases from the carbon atoms of the indolyl ring.

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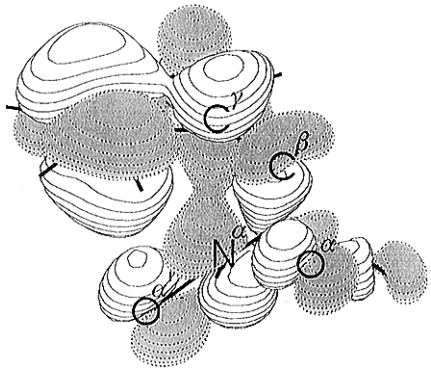
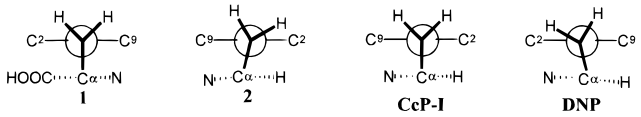


Figure 1. The highest (singly) occupied molecular orbital for **2**−diamide from a single-point unrestricted Hartree−Fock calculation at the B3LYP/6-31G(d) optimized geometry. In order to visualize with the program MOLDEN, the 3-21G basis set was necessary. The relative coefficients are unchanged by the basis set, however.

as an average 0.11 e is found on the alanyl chain (Table 1). Although experimental evidence for the predicted ASD is lacking, reported experimental spin densities for $TrpH^{\bullet+}$ in CcP compound I only total 0.84 e,[14] leaving unaccounted for 0.16 e.

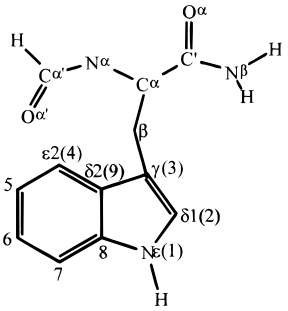
To test the validity of our isolated $TrpH^{\bullet+}$ as a model for $TrpH^{\bullet+}$ involved in peptide (amide) linkages of proteins, we also performed optimizations on the simplest diamides (substituting NH_2 for OH and CHO for H on the acid and amine, respectively) for **1** and **2**. This change did alter the dihedral angles of the alanyl chain and the spin density distributions, although approximately 0.1 e is still found on the alanyl chain. We also compared our calculated conformations to those found in the X-ray diffraction crystal structures of CcP[15] and DNP[16] and the Laue diffraction structure of CcP compound I (CcP-I).[17,18] Dihedral angles of conformation **2** are the most similar to the Trp conformations found in these enzymes with the C(O) trans to C^3 (C^γ) (cf. CcP-I and DNP). The considerably larger



spin density predicted for O^α of **2**−diamide than that seen in **2** seems incongruent with the carbonyl oxygen being oriented away from the indole ring. Figure 1, however, shows the singly occupied molecular orbital of **2**−diamide and provides a rationale as the lone-pair-type orbital on O^α exhibits positive overlap with the π orbital on N^α which also overlaps the π orbital on C^γ of the indole side chain.

Because the conformations of the two Trp residues which form radical cations in these enzymes were not identical to either **1** or **2**, we examined the experimental structures of CcP-I and DNP to determine other Lewis bases which might be within van der Waals distance of the indolyl π system. For CcP and CcP-I, two such atoms were located, the carbonyl oxygens of His-175 and Met-230, each within 3.06 Å of side chain carbons.[19] Although the carbonyl oxygen of Val-304 is close to the ring of Trp-306 in DNP, it is farther away than the oxygens in CcP and calculations on this system were not attempted. Optimizations {B3LYP/6-31G(d)} of indolyl radical cation with an amide carbonyl (as formamide) fixed in the same position as each of these two oxygens revealed *spin density delocalized onto the formamide oxygens*.

Proposed ET pathways in CcP include through-bond (covalent and H-bonds) and through-space paths, ending with a proposed transfer of the electron to the porphyrin from $TrpH^{\bullet+}$-191 through perpendicular π systems.[7,20] One proposed path for the electron transfer from cytochrome *c* to the heme of CcP includes a transfer from the sulfur of Met-230 to $TrpH^{\bullet+}$-191.[21] Our results suggest that the electron transfer pathway might



3--Trp-diamide with labelling

instead include transfer from Met-230 to $TrpH^{\bullet+}$-191 through the carbonyl oxygen of the Met residue. The reducing equivalent may then transfer from Trp-191 to the iron via the carbonyl oxygen of its proximal ligand, His-175, which is in direct contact with the Trp-191 π system.

In summary, our HF/DF study of $TrpH^{\bullet+}$ and its diamide form introduces the concept of anchimeric spin delocalization. We therefore suggest that magnetic resonance experiments to determine spin density distributions for amino acid radicals in proteins, particularly those seeking to unveil the mechanisms or pathways for LRET, consider ASD between side chains and the peptide backbone. The conformational dependence of ASD also lends support to the theory of conformational gating[22] of ET and suggests systems for study to detect conformational gating.

Supporting Information Available: Calculational details (4 pages). See any current masthead page for ordering and Internet access instructions.

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