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Coupling of the Amide I Modes of the Glycine Dipeptide

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Several approaches to calculate the coupling between the two amide I modes of the glycine dipeptide are compared. The full (ϕ, ψ) conformational space of the molecule is explored, and conditions for the validity of the different approaches are discussed.

It has been demonstrated recently that two-dimensional (2D) vibrational spectrosopy can be used to measure the coupling between amide I modes of small peptides.[1–7] Since this coupling is in some complicated way related to the geometry of the peptide backbone, one might hope to be able to determine peptide structures by measuring a complete set of coupling constants. A first, promising demonstration of this capability is the recent structure determination of the small peptide trialanine.[4,5] Since the measurement process of 2D-IR spectroscopy takes place on a ps time scale, this method has the prospective of being able to determine transient structures on the same, ultrafast time scale.

The most simple and very intuitive approach to relate the coupling constant to the peptide geometry is the transition dipole coupling (TDC) model,[8] which is based on two crude approximations: (a) the dipole approximation and (b) neglect of through-bond effects. It is well established that the amide I vibration (which is mostly the C=O stretching vibration) is accompanied by a charge flow between the carbonyl oxygen and the nitrogen which is responsible for about 1/2 of the total transition dipole moment of the amide I mode.[8] Hence, the size of the object which gives rise to the transition dipole (≈2.5 Å) is of the same order as the distance between adjacent amide I groups. As a consequence, the dipole approximation is extremely questionable. Furthermore, normal mode calculations on *N*-methylacetamide (NMA, CH_3–CONH–CH_3) show that the amide I vibration is not entirely localized on the peptide unit (–CO–NH–) and that methyl carbon and hydrogen atoms are involved as well.[2] Hence, through-bond effects will certainly contribute to the coupling between chemically bonded peptide units.

The most sophisticated way of calculating couplings between amide I modes would be from ab initio normal mode calculations of the complete polypeptide. With present computer power, this is feasible only for extremely small peptides, such as di- and tripeptides, but becomes impossible for larger polypeptides. Moreover, a structure determination will require some sort of optimization algorithm, which iteratively varies the conformation of a test molecule, calculates a set of coupling constants for each configuration and compares them with the experimental data. Since this will be a high-dimensional optimization problem (except for the smallest peptides) it will require many calculations of coupling constants, and it is necessary to seek for the least computer-expensive, but still sufficiently accurate methods to calculate a set of coupling constants for a given conformation.

In this paper, several approaches to calculate vibrational coupling constants are compared. These methods are increasingly sophisticated and (hopefully) accurate, but also increasingly computer-expensive. This is done for the glycine dipeptide (CH_3–CONH–CH_2–CONH–CH_3), the smallest possible peptide molecule with two peptide units. Its conformation can be described by one set of dihedral angles (ϕ, ψ), which also determine the backbone conformation of larger polypeptides. Except for the ab initio normal-mode calculation, the complete (ϕ, ψ) conformational space of the glycine dipeptide is explored, using four different approaches: (a) transition dipole coupling (Fig. 1, top-left panel), (b) interaction of transition charges (Fig. 1, top-right panel), (c) according to a semi-empirical PM3 molecular orbital calculation (Fig. 1, bottom left), and (d) according to a B3LYP ab initio molecular orbital calculation (Fig. 1, bottom right). The different approaches will be explained in detail in the next section.

Computational Methods

Transition Dipole Model: The transition-dipole coupling mechanism, which has been proposed initially by Krimm et al.,[8] is the simplest approach to calculate vibrational couplings. It assigns each peptide group a transition dipole and calculates the coupling constant according to

$$\beta_{ij} = \frac{1}{4\pi\varepsilon_0}\frac{\vec{\mu}_i\cdot\vec{\mu}_j}{r_{ij}^{\,3}} - 3\frac{(\vec{r}_{ij}\cdot\vec{\mu}_i)(\vec{r}_{ij}\cdot\vec{\mu}_j)}{r_{ij}^{\,5}} \qquad (1)$$

where the directions of the transition dipoles $\vec{\mu}_i$ and the vectors connecting two sites $\vec{r}_{ij}$ are properties which depend on the conformation (ϕ, ψ) of the peptide in an elementary way. The strength of the transition dipole is 0.374 D, it points towards the nitrogen atom with an angle of 20 ° with respect to the C=O bond, and its origin is located in between the carbon and oxygen atom with a distance of 0.868 Å from the carbon.

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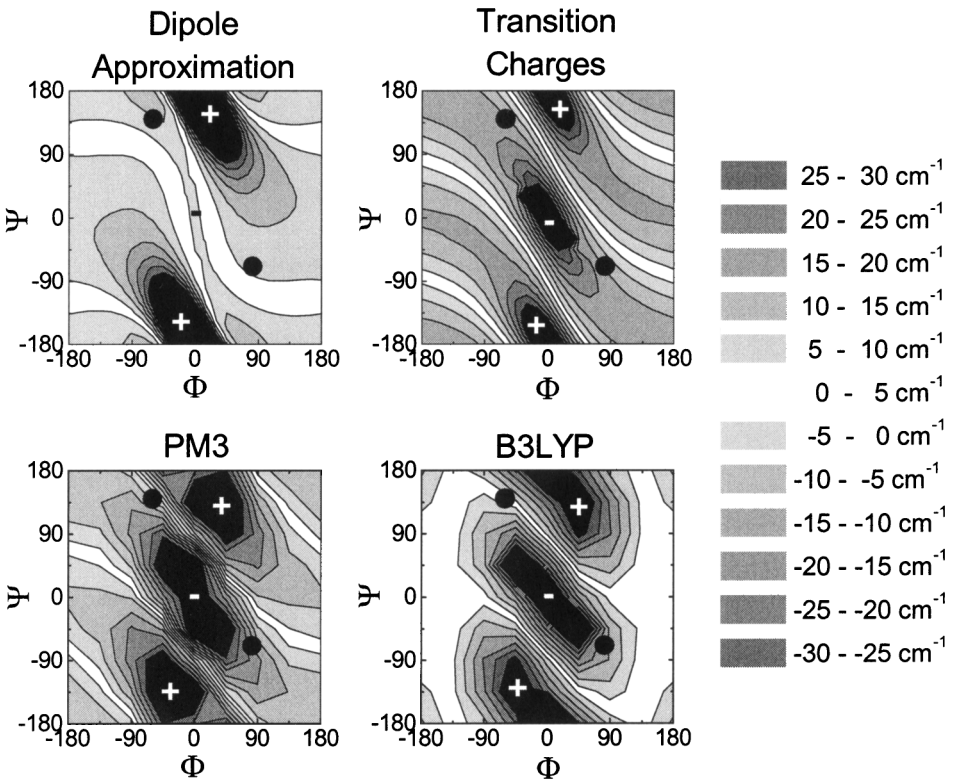

Fig. 1. The coupling between both amide I modes of the glycine dipeptide as a function of the dihedral angles ϕ and ψ, using transition dipole coupling (top-left panel), interaction of transition charges (top-right panel), a semi-empirical PM3 molecular orbital study (bottom left), and a B3LYP ab initio molecular orbital study (bottom right). Contour lines correspond to 5 cm^{-1} intervals. Regions where the couplings diverges are marked black. The C_7^{ax} conformation with (ϕ, ψ) = (82°, −69°) and the conformation of tri-alanine (ϕ, ψ) = (−60, 140), which has been determined recently using 2D-IR spectroscopy in connection with an ab initio molecular orbital calculation,[4,5] are marked by black dots. See text for details.

These numbers have been fine-tuned to best reproduce the IR spectra of regular polypetide structures.[8,9] However, the fact that the predicted coupling strength is very sensitive to the exact position of the transition dipole stresses that the dipole approximation is a rather crude one.

Transition Charge Model: To overcome the limitations of the dipole approximation, a set of transition charges has been calculated in Ref. 2. This approach still neglects through-bond effects as it assumes that the amide I vibration is completely localized on individual peptide units, and that the coupling is solely given by Coulomb interaction between the oscillating charges on the individual peptide units. In this approach, a point charge q_n and point charge flow dq_n are assigned to each atom n in peptide unit i, with position $r_n(x_i)$. The vibration of peptide unit i gives rise to an oscillating electric field which interacts with that of a second peptide unit j according to

$$\beta_{ij} = \frac{1}{4\pi\varepsilon_0}\frac{\partial^2}{\partial x_i \partial x_j}\left[\sum_{n,m}\frac{(q_n + dq_n x_i)(q_m + dq_m x_j)}{|r_n(x_i) - r_m(x_j)|}\right] \quad (2)$$

where x_i and x_j are the dimensionless normal mode coordinates of the amide I mode of peptide unit i and j, respectively. To calculate a set of transition charges, a density functional theory calculation (B3LYP level, 6-31+G* basis set, GAUSSIAN94[10]) has been performed on NMA, the smallest molecule with a peptide unit. After geometry optimization, vibrational eigenmodes, a set of Mulliken charges, and the first derivatives of the Mulliken charges with respect to the amide I normal mode were calculated. Partial charges flowing onto the methyl carbon and hydrogens were added to the corresponding C or N-atom of the peptide group. The strength (0.37 D) and orientation (25° to the C=O bond) of the transition dipole moments, also obtained from the transition charges, reproduce the experimental values very well, confirming the accuracy of the quantum chemistry calculation.

Partial Ab Initio Calculation: To at least partially account for through-bond effects, molecular orbital calculations have been performed. This approach still assumes that the amide I vibration is totally localized on the peptide unit (–CO–NH–) and neglects kinematic coupling through, for example, the CH bending mode of the C_α atom, which is the resonance closest to the amide I vibration. However, it allows for charge flows between peptide units and avoids the limitation of point charges. The calculations, which are essentially identical with those reported in Ref. 11, were performed as follows: At a given set of dihedral angles (ϕ, ψ) (with standard parameters for bond lengths and angles), both peptide units were displaced along the coordinates of the amide I mode. These coordinates were taken form the ab initio normal mode calculation of NMA described above. Only the atoms of the peptide unit (–CO–NH–) were displaced, while those of the C_α methylene group and the terminal methyl groups were fixed. The cou-

pling constant is computed according to:

$$\beta_{ij} = \frac{\partial^2}{\partial x_i \partial x_j} E(x_i, x_j) \quad (3)$$

where the energies $E(x_i, x_j)$were obtained from single point GAUSSIAN runs and the 2nd derivative was computed as a finite difference. Coupling constants have been calculated on two different levels, the GAUSSIAN semi-empirical PM3 implementation (Fig. 1, bottom left panel) and the B3LYP, 6-31+G* level (Fig. 1, bottom right panel).[10] It should be noted that the B3LYP calculation yields essentially the same map as that reported in Ref. 11, which was calculated on the Hartree–Fock 6-31(+)G** level on partially optimized structures.

Full Ab Initio Calculation: In addition, an (unscaled) normal mode calculation has been performed on the B3LYP, 6-31+G* level. This approach avoids all approximations mentioned above and is exact in as much as the quantum chemistry calculation is exact. Geometry optimization converged into the C_7^{ax} conformation with (ϕ, ψ) = (83°,−48°), which is the well-known vacuum structure stabilized by an intramolecular hydrogen bond. The coupling was deduced from the normal modes by making the following ansatz for the two-dimensional sub-Hamiltonian of the amide I modes:

$$H = \begin{pmatrix} \varepsilon_1 & \beta_{12} \\ \beta_{21} & \varepsilon_2 \end{pmatrix} \quad (4)$$

whose eigenmodes are

$$\phi_+ = \sin\alpha\cdot|1\rangle + \cos\alpha\cdot|2\rangle \quad (5a)$$

$$\phi_- = \cos\alpha\cdot|1\rangle - \sin\alpha\cdot|2\rangle \quad (5b)$$

where the mixing angle is given by $\tan 2\alpha = 2\beta_{12}/(\varepsilon_2 - \varepsilon_1)$, and can be deduced directly from the normal modes eigenvectors from the GAUSSIAN output using the C=O distance as a measure of the normal mode displacement. In the present case, we find for the mixing angle $\alpha = 13°$. Furthermore, this treatment also allows for a consistency check, as the same mixing angle is expected to be obtained for both amide I modes. The mixing angle differs by less than 1% in both cases, confirming that the amide I subspace is indeed separated from all other normal modes. Inverting Eqs. 4 and 5, one finds $\beta_{12} = -6.8\ cm^{-1}$ (see Table 1).

Discussion

The patterns in the Ramachandran plots of Fig. 1 exhibit some similarity, but differ significantly in detail. This holds in particular for extreme points such as (ϕ, ψ) = (0°,0°), where the dipole approximation yields a small negative value, while all others diverge with large negative couplings. This configuration is sterically impossible, since it would form a 6-membered rich with the oxygen of the first peptide unit and the amide proton of the second superimposed. It is nevertheless an instructive example to discuss what is happening: The transition dipole approach is not affected by the superposition of both atoms, since it assigns the transition dipole vectors of both peptide units to certain points close to the oxygen atoms, which do not come particularly close in the hypothetical (ϕ, ψ) = (0°,0°) structure. However, as the amide I vibration also involves the amide protons, a diverging interaction is obtained in all other methods which take into account the charges on the amide proton and its motion. The C_7^{ax} conformation is a non-planar distortion of the (ϕ, ψ) = (0°,0°) structure, forming a 7-membered ring with a hydrogen bond between the oxygen of the first peptide unit and the amide proton of the second. As summarized in Table 1, the dipole approximation still completely fails to predicts the coupling constant for this conformation (it even predicts the wrong sign), whereas all other methods reveal comparable results within ±35%. However, other points in the conformational range, such as the sterically allowed, completely stretched conformation with (ϕ, ψ) = (180°,180°) yields opposite signs of the coupling when comparing the B3LYP molecular orbital calculation with the less computer-expensive methods. Apparently, charge flows over the entire backbone (i.e. through bond effects) can no longer be neglected in the fully stretched conformation.

The conformation of tri-alanine, a molecule with the same central structure as the glycine dipeptide, has recently been determined using 2D-IR spectroscopy[4,5] combined with the result of an ab initio molecular orbital calculation.[11] From the experimentally determined coupling constant of $\beta_{12} = 6\ cm^{-1}$ and the angle between the transition dipoles $\theta = 106°$, we deduced for the conformation (ϕ, ψ) = (−60°,140°). In this region, all maps shown in Fig. 1 (except for the one using the dipole approximation) are comparable and would predict dihedral angles within a range of about ±20°. As yet, our structure prediction of tri-alanine has not been verified using established techniques.[13] However, explicit MD simulations have computed for the lowest free energy minimum (ϕ, ψ) = (−68°, 141°)[7,12], with ϕ only slightly smaller than our prediction (ϕ, ψ) = (−60°,140°). The transition dipole model, transition charge model and PM3 molecular orbital calculation, on the other hand, would predict conformations with even larger angle ϕ (see Fig. 1).

In summary, we conclude that the dipole approximation is too crude to be useful when aiming for quantitative results. The accuracy of higher level methods depends on the conformational region under study. As long as only a two-dimensional (ϕ, ψ) conformational space is to be explored, it is still feasible to assemble a map of ab initio normal mode calculation (2–3 CPU-days for each point with present computer power),[12] which takes into account all through-space and through-bond effects. Such a map can easily be parameterized. However, for a larger than 2-dimensional configurational space, this

Table 1. Comparison of the Coupling of the Amide I Modes of the C_7^{ax} Conformation Deduced from the Different Methods Described in the Text

	Dipole approximation	Transition charges	PM3	B3LYP	B3LYP Normal mode calculation
C_7^{ax} (82°, −69°)	+ 6 cm^{-1}	−9.4 cm^{-1}	−13.9 cm^{-1}	−10.6 cm^{-1}	−6.8 cm^{-1}

approach becomes impossible. Hence, a mixed strategy is proposed: Nearest neighbor coupling, for which through-bond effects are essential, should calculated on the highest available level, if possible using a pre-parameterized map. For peptide units that come close in space, but that are not adjacent in the polypeptide chain, through-bond effects are certainly much less important and cheaper methods, such as the approach using transition charges, can be used.

Finally, a general remark on the possibility to calculate coupling constants should be made: It is well known that any normal mode calculation overestimates vibrational frequencies by 5–10% because of electronic correlation effects and the neglect of anharmonicities of the potential surfaces. Hence, it is clear that even the ordering of the amide I states calculated from a normal mode calculation is completely arbitrary, since this would require an accuracy in the order of $< 1\%$ ($= 16\ \mathrm{cm}^{-1}$). Despite of this uncertainty, it is nevertheless realistic to calculate coupling constants in the order of just few cm^{-1}, much smaller than the error in the vibrational frequencies. This is because vibrational frequencies are computed from the second derivatives of the potential energy surface with respect to the nuclear coordinates, $\varepsilon_i = \partial^2 E(x_i)/\partial {x_i}^2$, whereas couplings are computed from the mixed second derivatives Eq. 3. One expects the *relative* error of both quantities to be of the same order of magnitude. Hence, coupling constants calculated from a quantum chemistry calculation can be compared with experimental results with some confidence, while vibrational frequencies are essentially arbitrary. This is why it is so important to have access to an experimental technique which can measure coupling constants separately from vibrational frequencies; 2D-IR spectroscopy can accomplish this.

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