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The Relative Mean-Square Displacement Amplitudes of Bonded Atoms; Evidence from X-ray Crystallography, Normal-Mode Analysis and Molecular Dynamics

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Abstract

The relatively rigid nature of covalent bonds imposes limits on the difference between the mean-square displacements of the bonded atoms projected in the bond direction Δ(m.s.d.a.). Evidence from X-ray crystallography shows that for many small-molecule structures this difference is usually less than the mean-square vibration amplitude of the bond. In less well ordered structures, where libration is more significant, molecular-dynamics calculations suggest that Δ(m.s.d.a.) values may be much larger. A model of the kinematics of a librating bond is proposed and it is shown that a first-order approximation to Δ(m.s.d.a.) is dependent on both second and fourth curvilinear moments.

Introduction

The relatively rigid nature of covalent bonds has been exploited in several ways in refining crystal structures and in investigating the mean-square atomic displacements derived therefrom.

Hirshfeld (1976) refined charge-deformation models of four small organic structures. He examined the mean-square displacement amplitudes (m.s.d.a.) of pairs of bonded atoms in the direction of the bond and showed that the difference Δ(m.s.d.a.) was smaller in the charge-deformation model than in that which assumed spherical atoms. By assuming that covalently bonded pairs of atoms should have effectively equal m.s.d.a.'s along their mutual bond, he was able to assert the superiority of the charge-deformation model. For atoms at least as heavy as carbon Hirshfeld estimated that the mean-square amplitude differences along bonds should normally be well under 0.001 Å^2.

Rollett (1970) stated that when two atoms are joined by a covalent bond, the difference between the atomic-vibration amplitudes in the bond direction could not exceed the amplitude of the bond-stretching vibration which for a carbon-carbon bond he estimated at 0.06 Å. He proposed that this vibration amplitude might be used to impose a restraint on the thermal parameters of bonded atoms in least-squares refinement. Terms representing the difference

between the components of anisotropic temperature factors in the bond direction might be included in the summation to be minimized with weights that would be the reciprocal of the square of the bond-vibration amplitude.

A similar idea was applied to macromolecular refinement by Hendrickson & Konnert (1980), first to isotropic and then to anisotropic m.s.d.a.'s (Konnert & Hendrickson, 1980). Using a riding model for bond dynamics, these authors restrained the variances of the bond lengths, and molecular dynamics was subsequently employed to evaluate this refinement strategy (Yu, Karplus & Hendrickson, 1985).

In this paper the relative m.s.d.a.'s of atoms joined by a covalent bond will be discussed in terms of a general model of bond dynamics which is applicable to small molecules and to macromolecules where much larger atomic displacements occur. The more specialized models such as the riding model (Busing & Levy, 1964) and the rigid-body model (Cruickshank, 1956; Schomaker & Trueblood, 1968; Pawley & Willis, 1970) of bond dynamics can be considered as special cases of the model proposed. The evidence from vibrational spectroscopy, X-ray crystallography, molecular dynamics and normal-mode analysis will be considered in order to characterize the types of motion available to covalent bonds.

Models of bond dynamics

Consider two atoms A and B, joined by a bond of instantaneous length d which has a mean-square bond vibration amplitude (m.s.b.v.a.) of σ^2. m.s.b.v.a.'s may be derived from vibrational spectroscopy and are determined largely by the atom types of A and B and the nature of the bond between them. At room temperature they are typically in the range $0 \cdot 001$–$0 \cdot 005$ Å^2 with larger values occurring for bonds involving hydrogen atoms (Cyvin, 1968).

In order to relate m.s.b.v.a.'s to Δ(m.s.d.a.)'s we may consider, without the loss of generality, the mean positions of A and B as lying on the z axis. Δ(m.s.d.a.) is then defined as

$$\Delta(\text{m.s.d.a.}) = \Delta^2 z_B - \Delta^2 z_A, \qquad (1)$$

where the m.s.d.a.'s of A and B projected onto the z axis are written as $\Delta^2 z_A$ and $\Delta^2 z_B$ respectively, and the corresponding root-mean-square values are written in the same notation without superscripts. These quantities may be derived from the Cartesian components of the conventional crystallographic m.s.d.a. tensor U by means of expressions such as

$$\Delta^2 z_A = n^T U_A n, \qquad (2)$$

where n is a matrix denoting a unit vector along z, and ^{T} denotes the transpose operation.

We wish to investigate the relationship between the crystallographic Δ(m.s.d.a.) and the spectroscopic m.s.b.v.a. In order to do this we need a model of the relative displacements of A and B. If the instantaneous length of the interatomic vector is d and θ is the instantaneous angle that the vector makes with the line joining the mean position of the atoms, then the variance of the projection of the bond onto the z axis is given by

$$\Delta^2 z = \langle (d \cos \theta - \langle d \cos \theta \rangle)^2 \rangle \qquad (3)$$

$$= \langle d^2 \cos^2 \theta \rangle - \langle d \cos \theta \rangle^2. \qquad (4)$$

If the length d and the angle θ are independent, then

$$\Delta^2 z = \langle d^2 \rangle \langle \cos^2 \theta \rangle - \langle d \rangle^2 \langle \cos \theta \rangle^2$$

$$= \sigma^2 \langle \cos \theta \rangle^2 + \langle d \rangle^2 (\langle \cos^2 \theta \rangle - \langle \cos \theta \rangle^2). \qquad (5)$$

The first term of the right-hand side of (5) is principally dependent on the m.s.b.v.a. of the bond while the second term is dependent on the nature of the bond libration.

The relation between the variance of the projected bond, $\Delta^2 z$, and Δ(m.s.d.a.) depends on how the motions of A and B are correlated. We introduce the correlation coefficient of the projected displacements defined as

$$r = \text{cov} (\delta z_A \delta z_B)/(\Delta^2 z_A \Delta^2 z_B)^{1/2}, \qquad (6)$$

where δz_A and δz_B are the instantaneous displacements of A and B. For bonded atoms r is always close to unity but it will be seen below that for a given $\Delta^2 z$ the value of Δ(m.s.d.a.) is highly sensitive to the precise value of r. In terms of this correlation coefficient

$$\Delta^2 z = \Delta^2 z_A + \Delta^2 z_B - 2r\Delta z_A \Delta z_B. \qquad (7)$$

The solution of (7) as a quadratic equation for Δz_B is

$$\Delta z_B = r\Delta z_A \pm (\Delta^2 z - t_A^2)^{1/2} \qquad (8)$$

where

$$t_A^2 = (1 - r^2)\Delta^2 z_A. \qquad (9)$$

Squaring (8) and subtracting $\Delta^2 z_A$ from both sides then gives

$$\Delta(\text{m.s.d.a.}) = \Delta^2 z - 2t_A^2 \pm 2r[\Delta^2 z_A (\Delta^2 z - t_A^2)]^{1/2}. \quad (10)$$

Equation (10) shows the possible values for Δ(m.s.d.a.) for given values of the projected m.s.d.a. of atom A, the projected m.s.b.v.a. of the bond and the correlation coefficient (r) of the projected displacements. For realistic vibrations of bonded atoms, only values of r close to unity yield real roots of (9) and hence meaningful Δ(m.s.d.a.). Obviously, the motions of bonded atoms must show a strong positive correlation in the bond direction.

Table 1. *Differences of mean-square displacements along bonds* $[\Delta(m.s.d.a.)]$ *and correlation coefficients* (r) *of projected atomic displacements along the line joining the mean positions of the atoms for two values of* $\Delta^2 z_A$

Values in parentheses show other Δ(m.s.d.a.)'s consistent with the associated correlation. These values have been derived from equation (10) and assume $\Delta^2 z = 0.002$ Å^2 which is a typical mean-square vibration of a single bond.

Model	$\Delta^2 z_A = 0.01$ Å		$\Delta^2 z_A = 0.1$ Å	
	$r \times 10^4$	Δ(m.s.d.a.) $\times 10^4$ Å^2	$r \times 10^4$	Δ(m.s.d.a.) $\times 10^4$ Å^2
Riding model (A riding on B) $r = \Delta z_B / \Delta z_A$	8944	−20	9899	−20
Zero-difference model $r = 1 - \Delta^2 z / 2\Delta^2 z_A$	9000	0 (−36)	9900	0 (−40)
Riding model (B riding on A) $r = \Delta z_A / \Delta z_B$	9129	20 (−47)	9902	20 (−59)
Totally correlated model $r = 1$	10 000	109 (−69)	10 000	303 (−263)

Models without libration

Two limiting cases of the proposed model may be distinguished. In the first case the bond libration can be neglected and (5) reduces to $\Delta^2 z \simeq \sigma^2$. This case may be relevant to the bond dynamics of many small molecules. An application of (10) to the case where $\sigma^2 \simeq \Delta^2 z = 0.002$ Å^2 is shown in Table 1. Such a value of σ^2 is typical of the variance of a carbon-carbon single bond. Table 1 shows several important examples. In the riding model of Busing & Levy (1964) an atom A is said to ride on atom B if it has all the translational motion of B plus an additional motion uncorrelated with the instantaneous position of B. For this model Δ(m.s.d.a.) $= \sigma^2$ and this may apply well to hydrogen atoms, which may be considered as riding on the heavier atom to which they are bonded. However, where pairs of atoms of roughly equal mass are bonded, the bond vibration is likely to be more equally partitioned between the bonded atoms and the zero-difference model may offer a better approximation. Indeed, a number of high-precision X-ray crystallographic studies have shown values of Δ(m.s.d.a.) of less than 0.001 Å^2 [see, for example, Seiler, Schweizer & Dunitz (1984)].

The totally correlated model is obtained by substitution of $r = 1$ into (10). This yields

$$\Delta(\text{m.s.d.a.}) = \sigma^2 \pm 2\sigma\Delta z_A \qquad (11)$$

for a bond with no libration, where $\sigma^2 = \Delta^2 z$. This equation gives the upper and lower theoretical limits for Δ(m.s.d.a.) given the m.s.b.v.a of the bond and the mean-square displacement of atom A. By substituting for Δ(m.s.d.a.) from (1) we have

$$\sigma = \Delta z_A \pm \Delta z_B. \qquad (12)$$

Taking the negative sign in this equation we have the limit given by Rollett (1970) and referred to above.

In the totally correlated model very large values of Δ(m.s.d.a.) may occur, as shown in Table 1, and although these are kinematically possible, the dynamics of molecular motion would suggest that

Table 2. *Values of* $\Delta(m.s.d.a.)$ *(Å × 10^4) for the nonapeptide oxytocin as determined from normal-mode analysis in Cartesian coordinates and from a* 1 ns *molecular-dynamics simulation*

Bonds involving hydrogen atoms have been omitted.

Bond type	Normal-mode analysis	Molecular dynamics
Main chain	2	229
Side chain	5	629

Δ(m.s.d.a.) values should be less than the m.s.b.v.a and should often conform to the original postulate of Hirshfeld (1976). The low-frequency normal modes which make the most significant contribution to m.s.d.a.'s leave bonds almost rigid. The few normal modes which give rise to significant bond stretching hardly contribute to the m.s.d.a.'s. This view is confirmed by normal-mode analysis (NMA) in Cartesian coordinates which operates within the harmonic approximation. Table 2 shows average Δ(m.s.d.a.) values for different bond types of the nonapeptide oxytocin. The NMA values for bonds are well within the limits of the riding model.

The librating bond

The second limiting case of (5) occurs when bond stretching may be neglected, in which case the equation reduces to

$$\Delta^2 z = d^2(\langle\cos^2 \theta\rangle - \langle\cos \theta\rangle^2). \qquad (13)$$

An important example of this case is rigid-body motion, which is particularly relevant in structures containing conjugated groups of atoms. Here the correlation between the librations perpendicular to the bond and the rigid-body translation parallel to the bond determine the value of r for each bond and hence the relationship between Δ(m.s.d.a.) and $\Delta^2 z$. This correlation may be derived from the tensor components in the TLS model of Schomaker & Trueblood (1968).

The model of a librating rigid bond may be generally applicable in macromolecular dynamics, where libration rather than bond stretching may be a significant contribution to Δ(m.s.d.a.). Unfortunately, it is difficult to test this hypothesis by extracting meaningful Δ(m.s.d.a.) values from X-ray diffraction studies of macromolecular structures. This is because the paucity of data gives rise to m.s.d.a.'s with large standard deviations. In the free anisotropic refinement of oxytocin (Wood et al., 1986) where data extended to a resolution beyond 1·1 Å, Δ(m.s.d.a.)'s were of the order of one standard deviation. In macromolecules such as proteins even larger errors would be encountered.

Estimates of root-mean-square librations can, however, be obtained from macromolecular structures by X-ray diffraction. The TLS model has been used by Howlin, Glover, Haneef & Moss (1987) in the refinement of many side groups in the protein ribonuclease. Root-mean-square librations of up to 12° were encountered in the side chains on the surface of the enzyme. Side-chain librations which could not be modelled could well be much larger.

We have used molecular dynamics (MD) to evaluate the sort of bond dynamics which may be taking place in larger molecular structures. In this procedure libration may be simulated and the harmonic model is not imposed. The MD simulation used the *SHAKE* algorithm (Ryckaert, Ciccotti & Berendsen, 1977) where bonds are constrained to have a m.s.b.v.a. of less than 10^{-5} Å^2. Δ(m.s.d.a.)'s from such simulations are therefore almost entirely due to libration and the values from a MD study of oxytocin (Treharne & Haneef, 1984) are shown in Table 2 where they can be seen to be two orders of magnitude larger than the corresponding NMA values. Further, Yu et al. (1985) found that their MD study of bovine pancreatic trypsin inhibitor was also incompatible with a riding model of bond motion.

Maclaurin expansion of (13) shows that even to a first approximation $\Delta^2 z$ is dependent on both second and fourth curvilinear moments and is given by the expression

$$\Delta^2 z \simeq \tfrac{1}{4}d^2(b_2 - 1)\langle\theta^2\rangle^2 \qquad (14)$$

where $b_2 = \langle\theta^4\rangle/\langle\theta^2\rangle^2$. For highly leptokurtic distributions large $\Delta^2 z$ values may thus correspond to modest root-mean-square librations. Unfortunately, it is not possible to propose one simple model of bond libration which is likely to account for the complex motions which occur in macromolecules. However, higher-moment assumptions are necessary before $\Delta^2 z$ values can be calculated.

The MD simulation of oxytocin (Table 2) suggests that large Δ(m.s.d.a.) values are also found in main chains. Fig. 1 shows the distribution of θ for a $C\alpha$–$C\beta$ bond in oxytocin during our MD simulation. For this distribution the root-mean-square libration is 10°,

$\Delta^2 z = 0.017$ Å^2 and Δ(m.s.d.a.) $= 0.010$ Å^2. Fig. 2 shows how large Δ(m.s.d.a.) values are generally associated with larger librations. $\Delta^2 z$ values are of the same order of magnitude as Δ(m.s.d.a.).

We have evaluated root-mean-square librations and $\Delta^2 z$ using

$$\langle f(\theta)\rangle = \frac{\int f(\theta)\exp\left[-V(\theta)/kT\right]\mathrm{d}\theta}{\int \exp\left[-V(\theta)/kT\right]\mathrm{d}\theta} \qquad (15)$$

where $f(\theta) = \theta$, θ^2, $d\cos\theta$, $d^2\cos\theta$ respectively. $\Delta^2 z$ was then calculated from (13). Typical anharmonic potential functions $V(\theta)$ such as

$$V(\theta) = \alpha[1\cdot0 - \cos(n\theta) + \beta\theta^2]$$

can yield values of $\Delta^2 z$ of the same order as that found in the MD simulation. Some examples are shown in Table 3.
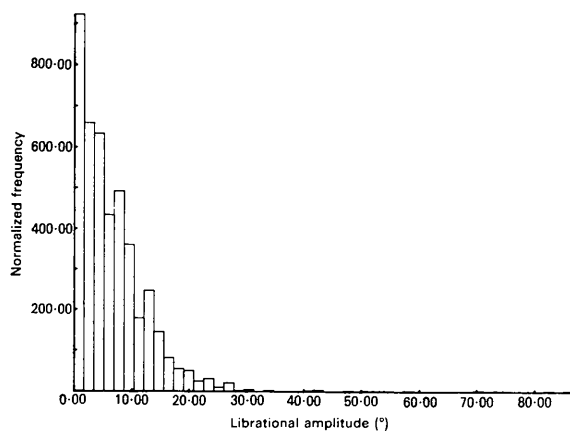

Fig. 1. Histogram of frequencies of librational amplitude of one $C\alpha$–$C\beta$ bond during a 1 ns molecular-dynamics simulation of oxytocin. The spherical polar angle plotted is that subtended by the bond to its mean position during the simulation. The frequencies in the bins have been normalized by division by the sine of the mean librational amplitude appropriate to that bin.
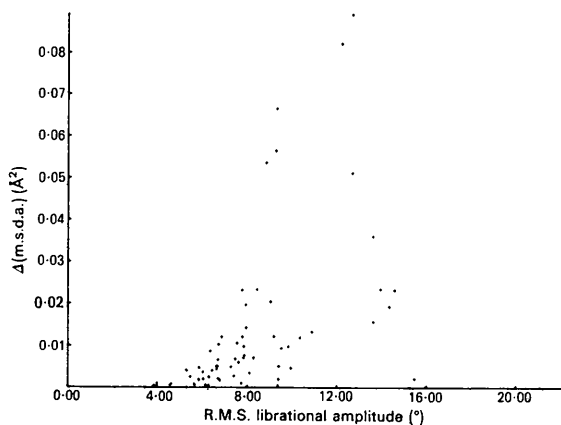

Fig. 2. Graph of Δ(m.s.d.a.) against root-mean-square librational amplitude for all bonds of oxytocin not involving hydrogen atoms. These values were derived from a 1 ns molecular-dynamics simulation of oxytocin.

Table 3. $\Delta^2 z$ values for a librating bond derived from equations (13) and (15) using anharmonic potential functions $V(\theta)$ as shown

In each function the value of α was chosen to yield a root-mean square libration of $10°$.

$V(\theta)$	$\Delta^2 z \times 10^4$ Å^2
$\alpha(1 \cdot 0 + 0 \cdot 1\theta^2)$	7
$\alpha(1 \cdot 0 - \cos 2\theta + 0 \cdot 1\theta^2)$	7
$\alpha(1 \cdot 0 - \cos 3\theta + 0 \cdot 1\theta^2)$	25
$\alpha(1 \cdot 0 - \cos 4\theta + 0 \cdot 1\theta^2)$	152

Discussion

X-ray crystallographic studies of small molecules show that Δ(m.s.d.a.)'s are normally much less than the corresponding m.s.b.v.a. Such values can be accounted for without invoking bond libration and can be understood from a harmonic model of atomic displacements. It should be noted, however, that high-precision studies are often carried out at low temperatures where some librational effects may be partially frozen out.

The evidence of molecular dynamics suggests that much more complex motions take place in larger more flexible molecules such as polypeptides and proteins and that Δ(m.s.d.a.) values may occur which are much greater than would arise from a molecule undergoing small harmonic vibrations. In view of the approximations involved in molecular-dynamics simulations and the lack of any internal assessment of errors, it would be wise to regard the results as yielding order-of-magnitude measures of mean-square displacements rather than quantitative estimates. Comparison with crystallographic refinements confirms that such an approach is reasonable (Northrup, Pear, McCammon, Karplus & Takano, 1980).

Rigid-bond tests which use Δ(m.s.d.a.) as an indicator of error in refined atomic displacement parameters should therefore be used with caution unless there is independent evidence which shows that bond librations are sufficiently small that they may be neglected. Moderate dynamic or static disorder as regularly occur in macromolecular structures (Frauenfelder, Petsko & Tsernoglou, 1979) may invalidate such tests.

Similar caution should also be exercised in using m.s.b.v.a. as a basis for restraining m.s.d.a.'s in

macromolecular refinement. In order to be valid such restraints should take into account the librational nature of bond dynamics and not rely on a simple riding-model approximation.

The applicability of rigid-bond tests could be further investigated using high-quality crystallographic refinements of small-molecule structures containing disordered groups. Higher-order models of libration (Prince & Finger, 1973; Shmueli & Goldberg, 1974) could be employed with better error estimations than are available either from macromolecular X-ray refinements or from molecular dynamics simulations.

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