Chirality and Computational Chemistry: A New Direction

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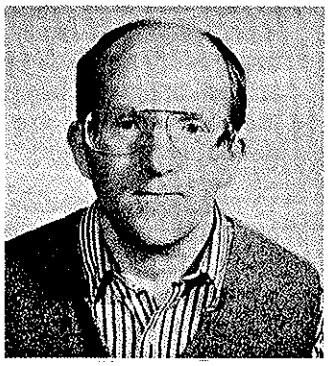

Abstract: Physical and biological responses to natural and synthetic chemicals occur only at molecular distances and at molecular dimensions. Computational chemistry calculates results in molecular dimensions, but has serious difficulties in accurately predicting chiral interactions between diastereoisomers and/or enantiomers. Prediction of structures and conformations for more complicated chiral interactions such as in protein folding and protein binding may be intrinsically inaccurate because the molecular consequences of the forces between these spatially close chiral centers are not explicitly taken into account. Molecular forces are calculated within and between molecules by optimizing the variables of distance, shape, and time. Symmetry in space over a distance is not the same as symmetry in space over a period of time. Thus for asymmetrical chemical structures, time distorts the mirror of chirality. The physical chemical properties of unequal population of *R*- and *S*- isomers of any compound are not directly predictable from those of binary mixtures containing equal amounts of opposite isomers. Only if the corresponding populations of *R*- and *S*- isomers are exactly equal can molecular properties be explained by time independent variables. Ignoring time-dependent variables inherently precludes understanding time dependent conformational and configurational changes which occur in xenobiotics, hormones, peptides and other chiral compounds which occur in an asymmetrical chemical environment.

All forces have a magnitude and a direction. Conversion of magnitude and direction into uniform molecular dimensions is the underlying principle of molecular biochemistry. Examining molecular forces in larger structures, as in molecular modeling, inherently concentrates uncertainty in the molecular forces among components within that structure. Reconstructing conformations of larger chemical structures from molecular forces measured on less complicated chemical structures, as in molecular mechanics, instead focuses the uncertainty in the force field parameters used in the calculations. A third approach is to link molecular forces to the time interval over which specific molecular events occur, as in molecular spectroscopy. Space, distance and time are three variables in computational chemistry used to quantify molecular properties. Each enables a separate focused analysis of molecular forces and structures to be correlated with experimentally observed physico-chemical, and/or biological properties. Interestingly, each approach also has inherent difficulties with directional and asymmetrical molecular forces.

Molecular Modeling: Direction Odd and Even

X-ray crystallographic data banks contain a fascinating collection of biologically relevant macromolecular structures of diverse space and size [1]. The scale in space is important because only objects viewed from the same space dimensions can be validly compared. The relative uncertainty in data accuracy and precision is a function of this same scalar grid.

The primary and fundamental strength of x-ray data is that its wavelength λ is in the 100 pm (1Å) range [2]. The length of carbon-carbon single bonds is about 150 pm (1.5Å). The interference pattern between the wavelength applied and detected enables the cartesian coordinates to be generated from x-ray structural analyses. The innate dimension of this array is the wavelength used to collect the data. Data must be collected from three directions (X, Y, and Z components must be included) for a three dimensional array to be constructed.

A useful way to visualize this cartesian array is as a series of cubes (dimensions $\lambda \times \lambda \times \lambda$) with "fuzzy" interfacial surfaces, arranged as rows and columns into a block the size of a single macromolecule. The "fuzzy" surface of each cube has a uniform finite thickness of uncertainty. This uncertainty contains three major components. The wavelength of the x-ray source is not exact. The mechanics and engineering of the x-ray instrument are not exact. The spatial position of each atom in the sample is not exactly uniform over the period of measurement. A rigid, spatially-defined structure is required to obtain molecular coordinates from an x-ray analysis. When molecular motion is localized, uncertainty is localized. All "fuzziness" and uncertainty is exactly spatial uncertainty and has physical dimensions relative to λ. Molecular modeling is a mechanism for visualizing molecules from atomic coordinates in their correct relative spatial dimensions without seeing the uncertainty.

As in a microscope or a telescope slightly out of focus, multiple sampling can be computationally combined to calculate a more precise focus than any

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single sampling. The danger is in assuming accuracy which is not present in the initial data. The peril of failing to label the size and location of uncertainty is that the research efforts itself loses focus. The most important uncertainty is in the boundary between what is known and what is unknown. Significant advances occur when the boundaries are irreconcilably different than previously expected.

Macromolecules must have rigid boundaries within a crystal lattice to obtain atomic coordinates from x-ray data. The problem with thinking of molecular dimensions as rigid in space is that understanding becomes stationary. A more serious error would be to assume that macromolecules are rigid in time.

Molecular modeling challenges the boundaries of X-ray crystallographic data. Extensive biochemical and biophysical research has been conducted on ion channels [3,4,5,6]. The publication in 1984 of the x-ray structure of the acetylcholine membrane receptor as five evenly spaced α-helices forming ion channel cylinders [7] has not significantly altered explanations of how ion receptors function. A cylinder is its structure and a cylinder is its function.

Structurally, each chain of an α-helix is composed of an N-H and O=C aligning step-wise up the helix and each loop contains 3.6 amino acids [8]. A result of the amides ordering in the helix is a dipole moment step-wise around the outside of each loop of the helix. The *R*- side chain could add to or subtract from this local dipole moment. The average dipole of each loop repeats loop after loop up the helix (Fig. (**1**)).

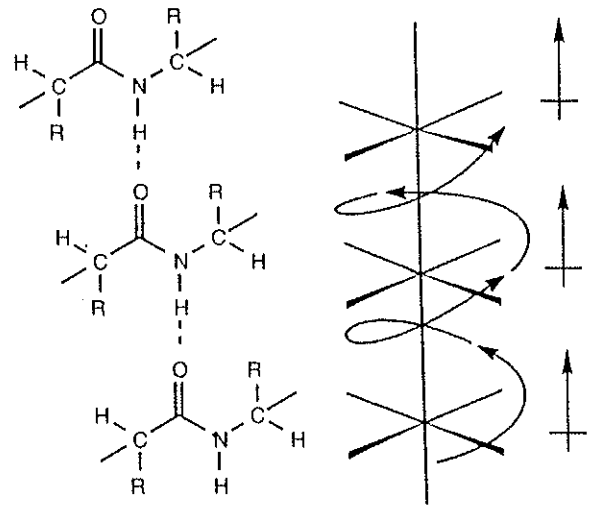

Fig. (1). The N-H and O=C amides align step-wise to form alpha helices. This alignment results in a repeating series of dipoles step-wise up the helix. The *R* side chain could add to or subtract from each dipole moment.

The net dipole moment of an even number of helices (of equal length) within a channel in and out of a membrane sums to zero. The cholinergic receptor has five chains in the channel; the β-adrenergic receptor has seven. A net dipole across the membrane occurs because only dipoles in opposite directions can cancel out (Fig. (**2**)). In forming a cylinder, two of the five (or of the seven) helices must align. Thus the dipolar force is twice as strong along one "face" of the cylinder. The positive ends of the dipole align at the bottom of the cylinder; the negative ends align at the top. This means not only is there a dipole moment parallel to the channel, but also a small dipolar force perpendicular to the channel.

None of these conclusions would be predicted from the assumption that the ion channel is a symmetrical cylinder. It has been postulated that the charged amino acid aspartic acid within the β-adrenergic receptor inhibits the flow of ions into the channel [9]. Even without a charged amino acid in the membrane helices, an odd number of chains would typically inhibit ion flow into the cell because the ion would have to pass through a dipolar field. Resistance to ion flow within the membrane would cause an electrical potential which can be measured at the cell surface. The electrical potential of a surface site outside of the cell may be much less important to the flow of ions than the electrical potential within ion channels. An anionic amino acid within the membrane channel would cause two dipolar moments, one pointing up the channel, the other down the channel. With a strong cation concentration such as that of Na^+ in the cell, the dipolar moment in the channel reverses direction.

The same properties which limits flow of ions would also limit the flow of dipolar compounds through the channel. In the absence of a strong cation, the dipole in the channel should stabilize the path of dipolar compounds like acetylcholine to the anionic site both from the inside and the outside of the cell membrane. A threshold increase in intracellular sodium ions would force some sodium into the channel, reversing the dipole direction and thereby releasing acetylcholine into extracellular space. On the loss of sodium from the cell channel, acetylcholine can return to the same site. Structural differences among the channel types could alter which specific dipolar chemical structures best fit within each membrane. Molecular features smaller than one loop of a helix could determine specificity in binding sites.

Assuming cylindrical symmetry in the channel would normally preclude consideration of that channel as an "active" site. When molecular forces are random, no one direction can be favored. In an α-helix, one end has the amide nitrogens pointing down, the other has the amide carbonyls pointing up. Directional forces over one helix are non-zero. Symmetry can therefore only occur if an even number of helices are considered. Since the terminal NH_2 of the membrane receptor begins inside the cell, an even number of receptors do not have a random dipole moment up the channel. The molecular forces in component structures almost always enable a more accurate understanding of the forces in the composite structure.

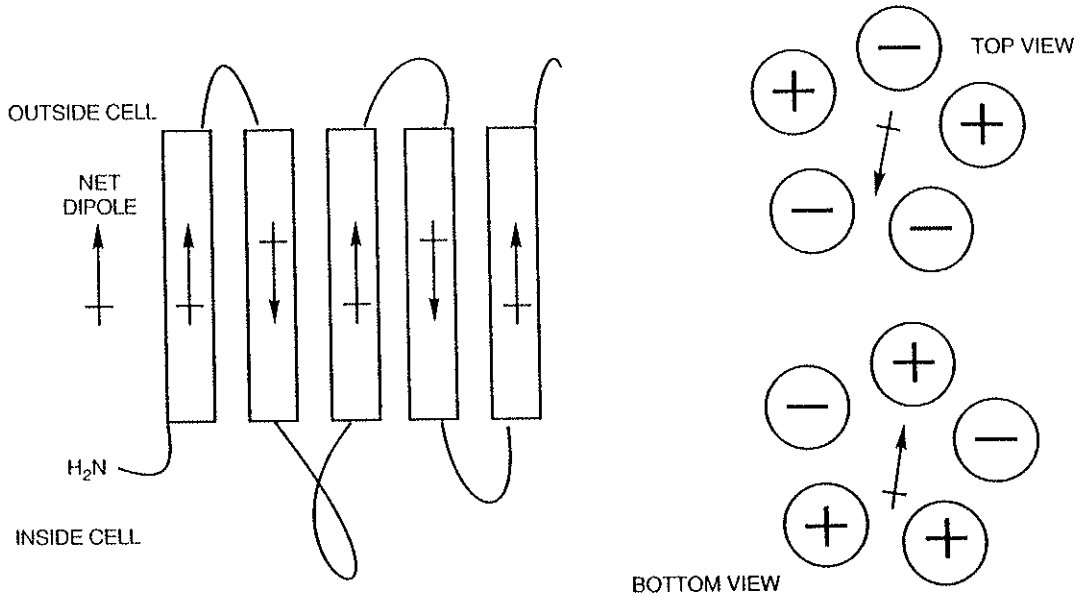

Fig. (2). The amide alignment forms a net dipole along each helix. An even number of helices (of equal length) has no dipole across the membrane. The dipole results from an odd number of helices. In forming a cylinder, three of the five dipoles align up the helix. This produces a small dipole perpendicular to the channel (its direction inverts between the top and bottom of the membrane).

Binding is always a time dependent variable in reversible reactions. A long term (time) association is not required for cholinergic or adrenergic binding to occur. Chemical structure is a better potential binding site than a rigid and chemically inert ion channel. Acetylcholine could "fill" the membrane channel lowering the effective dipole across the channel. The molecular dynamics of this arrangement is that the balance among dipolar forces can be rapidly restored when time dependent changes in dipolar forces occur. Compounds chemically different from the membrane can restore the static symmetry (no net direction in dipole moment within a period of time) but do not alter the spatial asymmetry (an uneven number of helices in a channel), nor the dynamic asymmetry (motion of the helix remains very slow relative to the motion of acetylcholine).

Only by viewing the individual components of an apparently symmetrical system can it be ascertained if its composition is symmetrical. When the outside of a ball appears symmetrical, it does not mean that its inside structure is also symmetrical. For example, a mixture containing an even number of asymmetrical pairs (matched mirror images) is exactly symmetrical. To create the whole as a summation of its parts, the parts within the whole, as well as the whole, must be systematically examined.

Molecular Mechanics: Paths of Symmetry

The forces which exist between atoms within molecules compose the atomic distances which define molecular structure. A force-distance curve describes the optimum (most likely, most stable) distance between any two atoms (Fig. (**3a**)). Molecular mechanics programs calculate distances between atoms in more complicated chemical structures using force constants obtained from physical measurements of distances in chemically simpler structures [10,11,12]. A single point corresponds to the energy minimum of the force-distance curve, and the focus of computational chemistry is towards calculating that distance. A second order parabolic equation (a harmonic oscillator) can approximate this function, but third order and/or fourth order components are often included to increase accuracy. The slope of the force-distance curve is steeper for atoms moving towards each other than for those moving away from each other, i.e. it requires slightly more force to move two atoms some distance closer to each other than to move them the same distance away from each other. The smaller the distance moved, the more alike the magnitude of movements closer and away will be. The mean time independent distance between them is a constant. Since there is a force-distance curve for each set of two atoms, finding the optimum distance among all sets of two atoms forms a precise molecular shape.

An interesting consequence to this approach is that same answer in distances among atoms is obtained averaged over a very long period of time as that selected over a very short period of time. The mean distance is obtained in the first case since larger motions left and right balance over time to near the average distance, but larger motions are less frequent than smaller motions. Smaller motions left and right balance over time to nearer the average distance. The longer the time period over which the motion is averaged, the more important smaller and smaller

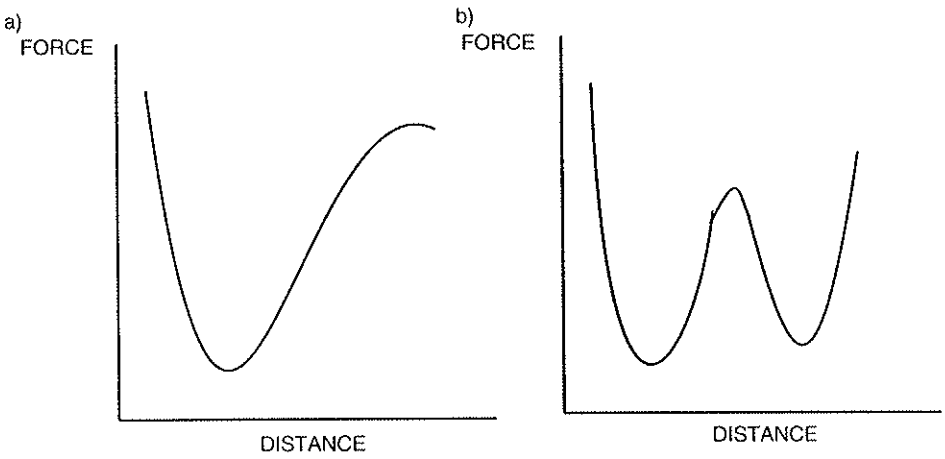

Fig. (3). The force between any two atoms is repulsive when they are too close, but attractive when they are too distant. The distant. The distance at lowest energy is calculated by molecular mechanics: a) one energy minimum; b) two energy minima.

motions become, and correspondingly the more precise the position of each atom. Molecular mechanics assumes a vapor phase, i.e. each molecule functions independently of each other molecule. Freezing molecular motion for a very short time interval and viewing only one molecule selected at random, distances are precise and rigid. Slower motion would appear as some fuzziness (small imprecision) in the more mobile distances. Accuracy is obtained by averaging the distances of a small number of molecules to assure that the distances were representative of the molecules in the sample. Selecting a very small time interval insures no large variation in distance has time to happen.

Two fundamental concepts inherent in this approach however must be considered. First, the actual confidence intervals for distances between atoms are not based on precision, but on accuracy. The distances calculated between atoms can never be more accurate than the techniques used to measure them and/or the parameters used to calculate them. Accuracy in the position of atoms within a molecule must be reliable enough to determine if the object in the "picture" moved while observed. It is conceptually important to "freeze" molecular motion (e.g. in a very short time frame of reference) upon which molecular motion can be added and from which molecular dynamics begins. However assuming more accuracy is present than actually exists biases and undermines any conclusions based on the results.

Secondly, although a single point is the energy minimum of the force-distance curve, experimentally the force-distance curves sometimes have more than one minimum (Fig. (**3b**)). NMR demonstrates that there are compounds (e.g. N,N-dimethylacetamide) which can exist at equilibrium as more than one conformation during the period of observation [13,14,15]. Two different distances can have similar energy minima with an energy barrier to interconversion between them. A fundamental difficulty in understanding both the part and the whole follows from this result. The average distance of both states is not the actual distance in either half. Determining the optimum distances in only one half the data, even if known with great accuracy, does not erase the fact that half the data had been discarded. A duplicate line of reasoning follows when the two energy levels are similar, but not identical. (If the conformer ratio is 55/45, the maximum accuracy that can be achieved with only one conformation is 55%.) Conformational analysis provides essential information whenever the parts do not coadd to the whole.

Molecular mechanics computationally confirms (consistent with NMR and X-ray crystallographic data) that the two methine protons in pseudoephedrine are in a trans position; in the case of ephedrine (Fig. (**4**)), NMR and x-ray crystallographic data have determined that in ephedrine the methines are cis to each other [16,17,18], but molecular mechanics predicts the methines are trans. There is no obvious mechanism by which the cis conformation of the methine protons in ephedrine are energetically favored in solids and in both aqueous and non-aqueous solution. Two force-distance solutions must exist for the same atoms attached to the same molecular sites, one for pseudoephedrine, one for ephedrine. Altering the relative chiral direction of any one site in an *R,R-* isomer to an *S*-isomer somehow causes a conformational change in which half the molecule rotates 180°. Why does molecular mechanics disagree with the observed spectroscopic results?

One potential answer is that molecular mechanics calculations are based only on time independent parameters. Chiral compounds racemize slowly over time, which means configurations are always time dependent variables. Conformational changes could in principle precede configurational changes. Thus time independent forces may also fail to adequately explain the conformations which exist in chiral compounds. Time dependent parameters are not used to characterize diastereoisomers and enantiomers [19], which includes the parameters for L-amino acids and for

more complicated combinations of amino acids like peptides.

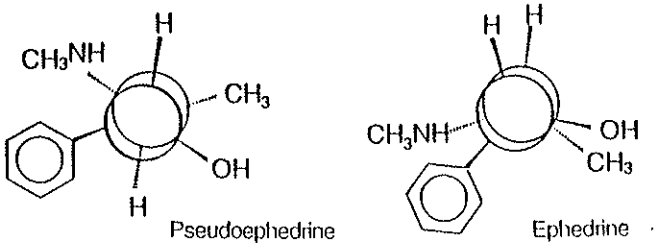

Fig. (4). The two methine protons are in a trans position in a pseudoephedrine but in a cis position in ephedrine, by x-ray crystallography and NMR spectroscopy. In contrast, molecular mechanics predicts that each set of methine protons is in a trans position.

A way of visualizing time dependent forces is a person throwing a boomerang. Initially and finally, the object's time independent position is in the hand that threw it. A harmonic oscillator can explain motion away from the hand and back, but its time dependent position is not the same as the time independent position. A time dependent perturbation occurs at a specific time and disappears at a specific time. A critical element and the chemical basis for an enzyme deteriorating with age may be time dependent perturbations of its structure, or time dependent changes in the structures which make the enzymes. The rate at which two *S*-amino acid molecules become one *S*-isomer and one *R*-isomer (racemization) is slow on the biological time scale, but like all rates is a time dependent physical constant. The initial asymmetry is a time dependent perturbation. The effect of this time dependent perturbation remains intact until symmetry reoccurs. The variables of shape and distance alone cannot account for time dependent occurrences. For a precise understanding of chirality, the time variable must be definitively included.

Molecular Spectroscopy: Time and Direction

Molecular spectroscopy from its fundamental principles (unlike molecular modeling or molecular mechanics) is explicitly connected to time. Excellent review treatises on spectroscopy are available [20]. Quantum chemistry is conceptually and mathematically elegant, especially in determining molecular properties related to time and distance.

The smallest time interval during which an event occurs at the molecular level defines that event. For example, a CH_2 wag back and forth occurs 3.97×10^{14} times in one second (i.e. at a frequency of 3.97×10^{14} Hz). The single molecular event occurs in 2.52×10^{-15} s. On lowering the temperature several degrees, the average bond distance decreases and fewer motions occur during the same interval. A shorter time (eg. 2.48×10^{-15} s) also corresponds to a smaller average distance (wag) back and forth. Shorter and longer distance of length can be translated into slower and faster units of time.

All molecular phenomena can be divided into individual events which reappear again and again exactly the same, time after time. Observation of these properties can be thought of as viewing motion through a stroboscope. Changes which reoccur once during an interval of the stroboscope are observed as unchanging because they vary at the same rate as the window of observation. The "length" of the time window determines the energy that can "fit" into that window. A period of 3×10^{-9} Hz corresponds to molecular transitions in the nuclear magnetic resonance frequency range (1.2 mJ/mole or 0.3 mCal/mole); a period of 1×10^{-13} Hz corresponds to molecular transitions in the infrared frequency range (12 kJ/mole or 3 kCal/mole) [21]. The window of 12 kJ/mole is also the same energy transition range as the binding of diastereoisomers [22] and ligand binding of proteins [23].

Measuring frequencies is potentially complicated because the time intervals of the frequencies sent and received are similar. According to the Nyquist theorem [24], one must sample at least twice during any interval to know if an event occurred once during that interval. In molecular spectroscopy, one has no information whatsoever in an interval smaller than the period of observation. One must judiciously sample at least twice in an interval to obtain directional information. Understanding direction in time is a prerequisite for identifying time dependent parameters involved in chemical structures and conformations.

Spectroscopy requires accurately measuring time. A fundamental and unavoidable problem is that symmetrical time is different than asymmetrical time. A stopwatch with a second hand can be used to measure time. Symmetry is correct when time does not depend upon direction because 60s clockwise (CW) exactly equals 60s counter clockwise (CCW) (Fig. (**5a**)) and when at the half interval, 30s CW equals 30s CCW (Fig. (**5b**)). Frequencies are time intervals (1/t) and 30s is one half the 60s time period. Time independent frequencies are symmetrical.

Time dependent frequencies are asymmetrical at all times except the full or half interval. The concept parallels the solution of the quantum mechanical problem of charge density of a time dependent particle in a box [25]. The maximum asymmetry occurs at 1/4 and 3/4 interval: the second hand at 15s using a CW clock occurs at a different spatial position than 15s on a CCW clock. Since the difference in time between 15s and -15s time vectors is 30s (i.e. half the interval), the magnitude of the asymmetrical component is as large as the symmetrical component of time. The symmetrical component of time follows the cosine function; the asymmetrical component follows the sine function.

Spectroscopic attempts to measure appreciable differences between left handed and right handed frequencies have not been successful [26]. Measurements were taken at the whole (all *L*, or all *D*) and the half interval (half *L* and half *D*), not the 1/4 or 3/4 interval. The argument has been presented that due to parity, the asymmetrical component of circular dichroism signals must sum to zero [27]. When *R* and *S* isomers are present at a 1/4 or 3/4 ratio, parity does not exist in a solution mixture nor in the solid formed on solvent evaporation. Symmetrical frequencies cannot explain the spectral properties of mixtures in which isomers are present at unequal mole ratios. Neither does parity exist when only one isomer is present. Symmetry is a glass half full equals a glass half empty. Asymmetry is a glass 3/4 full equals the same glass 3/4 empty. Selecting a frame of reference is deciding to follow either the air or the water.

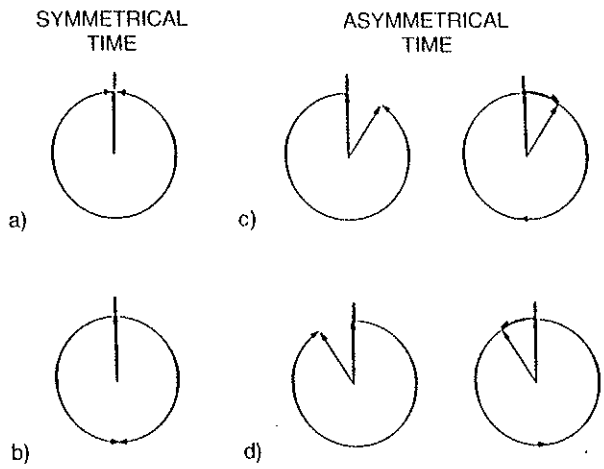

Fig. (5). When clockwise (CW) and counterclockwise (CCW) times coalesce equally, time is symmetrical. (a) 60s CW equals 60s CCW; b) 30s CW equals 30s CCW. All other times are asymmetrical; c) 66s CW equals 54s CCW and d) 54s CW equals 66s CCW each cycle at precisely the same time over and over again.

An equimolar mixture of an *R,R*-isomer with an *R,S*-isomer contains precisely the desired 1/4 and 3/4 mole ratio at which asymmetrical chiral interactions should be spectroscopically detectable. The solid state NMR spectra of an equimolar mixture of *R,R*-pseudoephedrine with *R,S*-ephedrine is different from an equimolar mixture of *R,R*-pseudoephedrine with *S,R*-ephedrine [28]. In contrast if the net force were in fact directionless, inverting the direction of half the components would change nothing.

Interestingly, the unit cell in X-ray crystal structure of *R,S*-ephedrine has 3/4 of the isomers in one conformation, the fourth in an opposite conformation [29]. It is impossible to divide the unit cell into symmetrical halves. For an equimolar mixture with pseudoephedrine, the problem is which two of the four molecules in the unit cell are replaced with the *R,R*-isomer. There is no symmetrical answer. Except for the racemate, asymmetry is fundamental to any explanation of the orientation and/or organization of these isomers.

Likewise in the solid state, NMR spectra of an equimolar mixture two amino acids, (the first an *S*-isomer, the second an *RS*-isomer), were very different from the same equimolar mixture in which the second was an *S*-isomer and the first an *RS*-isomer [30]. Frequencies corresponding to large conformational changes are observed which cannot be assigned to unique chemical structures. Chemical structure between the sets is identical except that in half the structures, chirality is reversed.

Corresponding results occur in the infrared frequency range [31]. Frequencies are observed which cannot be assigned to unique chemical structures. Chemical structure between the sets is identical except that in half the structures, chirality is reversed. If the components were symmetrical, inverting the chiral direction of half the components would alter nothing. Using infrared vibrational circular dichroism (VCD) small directional components have been found in the infrared spectra [32]. The attempts again were to find (match) the spectra of *R* and *S* (or (+) and (-)) isomers. Beginning with half *R* and half RS, the asymmetrical component of frequencies must be unequal.

Asymmetry in spectroscopy occurs because the (time) frame of reference has a direction (i.e CW or CCW). Asymmetrical CW and CCW frequencies do routinely and repeatedly occur at the same position in time: 54s CCW always exactly equals to 66s CW (Fig. (**5c**)). In contrast even though 54s CW always exactly equals to 66s CCW (Fig. (**5d**)), neither equals 54s CCW. Collection of data over a longer time interval (time averaging) does not alter the result because N x 54s CCW is spatially indistinguishable from N x 66s CW. The slower time and the faster time traveling in opposite directions always match spatially within the interval, even though the length traveled on the two paths are constantly not equal (54s directionless time never equals 66s directionless time).

Unless one observes the stopwatch a "second" time during the interval (consistent with the Nyquist theorem), it is impossible to know whether a specific path is CW or CCW. (If the reference clock speed were increased to 66s/min, 54s CCW would instead coalesce with the 78s CW time, but also 60s CW would now equal 72s CCW.) Choosing the "second" time at the half interval would be counterproductive. The results are time dependent because the directional components can be detected only when measured from a CW (or a CCW) frame of reference. The vector sum and/or difference between any two asymmetrical frequencies has no unique solution unless the sign of direction of each of the components is known. CW (and CCW) frequencies and corresponding directional forces can coadd in a corresponding direction. In the absence of information on their relative directions in individual frequencies, the resultant effects from combining vector components are limited to scalar interactions.

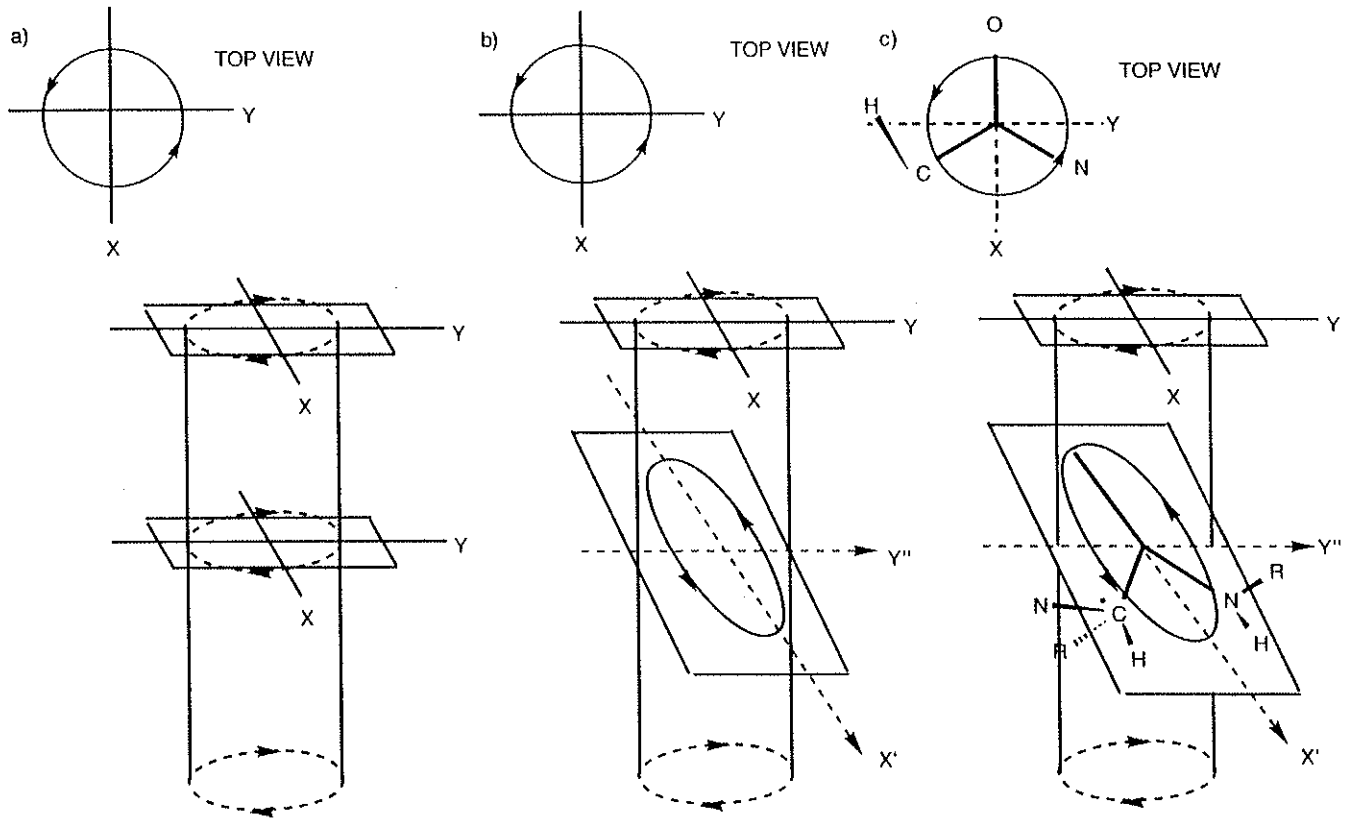

Fig. (6). All frequencies are circular, a) perpendicular to the time axis. Ellipticity b) results from viewing a constant circular frequency from a skewed frame of reference (the viewer is not perpendular to the time axis). A peptide sp^2 amide bond c) forms a circular plane and the C,O, and N conformation is either CW or CCW to a time axis. The angle is skewed relative to the circular dichroism frequency from *C when its axis is not perpendicular to the same chiral frame of reference. All angles and directions should be relative to a common time axis.

On a 60s clock observed once a minute, 66s CW (faster) equals 54s CCW (slower). Assume of two fast clocks (66s CW), a random process causes one of them to instantaneously lose direction and becomes a slow clock 54s CCW. No change in position would be observed between the two events; but the average time lost in the interval would be 12s. In contrast if time were symmetrical, a 60s CW clock would lose no time if it instantaneously became a 60s CCW clock. The time dependent loss of 12s is real in a symmetrical frame of reference. If time t has direction, frequency 1/t has direction. It is a serious conceptual and accounting error to ignore frequencies and corresponding energy levels which are time dependent.

Three distance dimensions X, Y, and Z describe chirality, but so do X, Y, and $1/t$ in which t is the time variable. The absolute configuration of a chiral molecule can be determined by X-ray crystallography; frequencies in the short UV range (10^{15}Hz) related to chirality are typically measured by circular dichroism (CD). Carbonyl absorbance is also in the same spectral range. The theory of circular dichroism usually does not correspond to the spectra observed experimentally and the concept of ellipticity is introduced to explain these results [33, 34].

All other frequencies are exactly circular and a circle moving along a time axis is cylinder (Fig. (**6a**)). An ellipse is formed by passing a plane at any skewed or obtuse angle through a cylinder (Fig. (**6b**)). Every plane perpendicular to the cylinder identifies the identical circular frequency and every different angle of skew reflects a precise position of the viewer within the chiral frame of reference. One can always view this circular frequency by observing perpendicular to the time axis. Otherwise the frequency observed depends upon the position of the observer within the chiral frame of reference.

When more than one chiral frequency exists, a single chiral frame of reference is required in which their magnitude and direction can be compared. (To know if a clock is fast or slow, one must have reference clock). A chemical structure in which two chiral frequencies exist is a peptide. An sp^2 amide bond (Fig. (**6c**)) forms a circular plane and the C, O, and N conformation is either CW or CCW to a time axis. The time dependent conformations are chiral because the CW and the CCW conformation are not superimposable. The angle is skewed relative to the circular dichroism frequency from the chiral α-carbon when its axis is not perpendicular to the same chiral frame of reference.

The chiral methine proton can be up or down from the plane. Up and down in a time frame of reference corresponds to CW and CCW directions. The carbonyl π-bond has an explicit positive and negative direction [35] again relative to the plane of the amide. Both are time dependent phenomena. Entropy requires that over time from any initial configurational direction (*D* or *L*, *R* or *S*, (+) or (-)) at the chiral center within a peptide, half the methine protons will invert direction. Since this slow process can take longer than decades, the state of the molecule under observation is much closer to the initial asymmetrical conditions than the final racemized state. Entropy requires that over time from any initial orientation (direction up and down) in the π-bonds within a peptide, half will flip direction. No time independent order has permanence.

Ellipticity is strong evidence that two frequencies are linked in time to the same chiral frame of reference. A chiral center next to an amide bond could initiate the original direction within the π-carbonyl bond and/or slow the rate at which it flips. The rate at which the two frequencies remain linked over time could be fundamentally linked to degradation. Conformational changes could occur after the frequencies are no longer linked (and before configuration changes at a chiral center takes place). As the number of linkages decrease and the number of sites increase, the peptide molecule should become less ordered and more amorphous. A peptide may be at its optimum conformation at a specific "age" corresponding to a specific degree of linking at specific sites. The linkage of two frequencies could also in principle be reinitiated, strengthened or weakened by external sources such as chiral sites within chiral molecule. The specific angle between the chiral methine proton and the adjacent planar amide group along the peptide designates a precise secondary peptide conformation. Definitive detection of these angles in solution is possible by observing the sign (and magnitude) of magnetic coupling stepwise "walking" up the ^{15}N and ^{13}C isotopically labeled peptide backbone using with multi-dimensional NMR experiments [36]. These time dependent nuclear magnetic frequencies, unlike time dependent circular dichroism frequencies, are far too weak to form or stabilize specific peptide conformations.

Time dependent molecular forces which link the chiral center and the amide plane are a primary and unacknowledged mechanism which can define how and why the peptide backbone folds and unfolds. Prediction of sites and mechanisms by which chiral molecules, such as xenobiotics, hormones, and/or environmental toxins, bind is different from a symmetrical than an asymmetrical frame of reference. Time independent forces fail to explain any time dependent phenomena. The reason an unequal population of chiral compounds initially occurs is a more complicated question [37]. Once populations of asymmetrical molecules are unequal, chiral forces within a space, distance and/or time are never balanced until racemization over time restores equal populations between opposite isomers.

Conclusion

Chiral molecular forces are pairwise (eg. left-right) balanced at equimolar concentrations. Symmetry and parity arbitrarily divide the dimensions of space, distance, and/or time into an even number of component parts. Whenever a localized space, distance, or time contains an uneven number of component parts, symmetry and parity is precluded. Whether it is the number of helical chains within a channel, the average distance travelled in a harmonic oscillator, or the number of frequencies which can fit in a time interval, an odd number of unit steps can never fit evenly into an even number of corresponding spaces. Nor can an even number of unit steps fit an odd number of corresponding spaces. The fundamental flaw with most theories of chirality is that time dependent phenomena cannot be directly predicted from time independent phenomena. Enantiomers and distereo- isomers are both time dependent. Discerning what happens in the presence of 2,4,6..... chiral centers does not predict what happens with 1,3,5..... chiral centers.

Chirality and asymmetry cannot be adequately understood except from a time dependent frame of reference. Whether a chiral compound is a xenobiotic, a hormone, or an environmental toxin, the molecular forces which define the conformation must have a chiral frame of reference. In the case of symmetry, no specific chiral frame of reference exists. Whether the binding sites are peptides, carbohydrates, or other local chemical environments, attempts at identifying its association with a chiral compound requires a common time dependent chiral frame of reference. With asymmetry, it is not just space, but space in time that matters.

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