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Visualization and comparison of molecular dynamics simulations of leukotriene C_4, leukotriene D_4, and leukotriene E_4

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Molecular dynamics simulations of leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and leukotriene E_4 (LTE_4) were carried out, and the data were visualized in an animated video format. Three-dimensional ghost images show the positions of the heavy atoms of all three molecules throughout the simulations. The ghost images can be superimposed to give a single three-dimensional image in which the shapes of the most populated conformers of each molecule are apparent and can be compared. Leukotriene D_4 was found to occupy mostly T-shaped conformations, while LTC_4 occupied mostly cup-shaped conformations, and LTE_4 occupied a wide range of conformations spanning the LTD_4 and LTC_4 types. Digital filtering and graphing of the internal geometries of the molecules as a function of time revealed differences in dynamic behavior. The results are discussed in light of current knowledge about leukotriene receptors.

Keywords: molecular dynamics, visualization, comparison, animation, videotape, digital filtering, cysteinyl leukotrienes, LTC_4, LTD_4, LTE_4, leukotriene receptors, receptor antagonists

INTRODUCTION

There is considerable interest in the discovery of cysteinyl leukotriene receptor antagonists. Evidence has been accumulating that the cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 (Figure 1) are responsible for some of the symptoms of asthma,[1] and clinical studies have shown that cysteinyl leukotriene receptor antagonists are able to reverse bronchoconstriction induced by exercise or allergens in asthmatic patients.[2] There is also evidence that the cysteinyl leukotrienes may be involved in other disease states.[3]

Information about the three-dimensional structures of the cysteinyl leukotrienes or their receptors would be useful in designing novel receptor antagonist structures; however, no X-ray crystal structures of the cysteinyl leukotrienes have been reported. Nuclear magnetic resonance (NMR) studies of these molecules have provided only limited structural information,[4] and three-dimensional structures of cysteinyl leukotriene receptors have not been reported. In this commonly encountered situation, molecular modeling can sometimes provide useful three-dimensional structural information. Methods such as systematic conformation searches,[5] molecular dynamics,[6] simulated annealing,[7] distance geometry,[8] and Monte Carlo sampling[9] can generate numerical output that contains information about many conformations of a complex molecule. New programs for the analysis and visualization of molecular dynamics simulations have been reported.[10] Digital filtering techniques have been used to simplify the motions observed in molecular dynamics simulations and to reduce the volume of data that must be analyzed.[11] Cluster analysis has also been used to simplify dynamics output by grouping the conformations occupied during a simulation into families.[12]

In the study reported here, 150-ps molecular dynamics simulations of LTC_4, LTD_4, and LTE_4 in water at physiological temperature were carried out and the data were ex-

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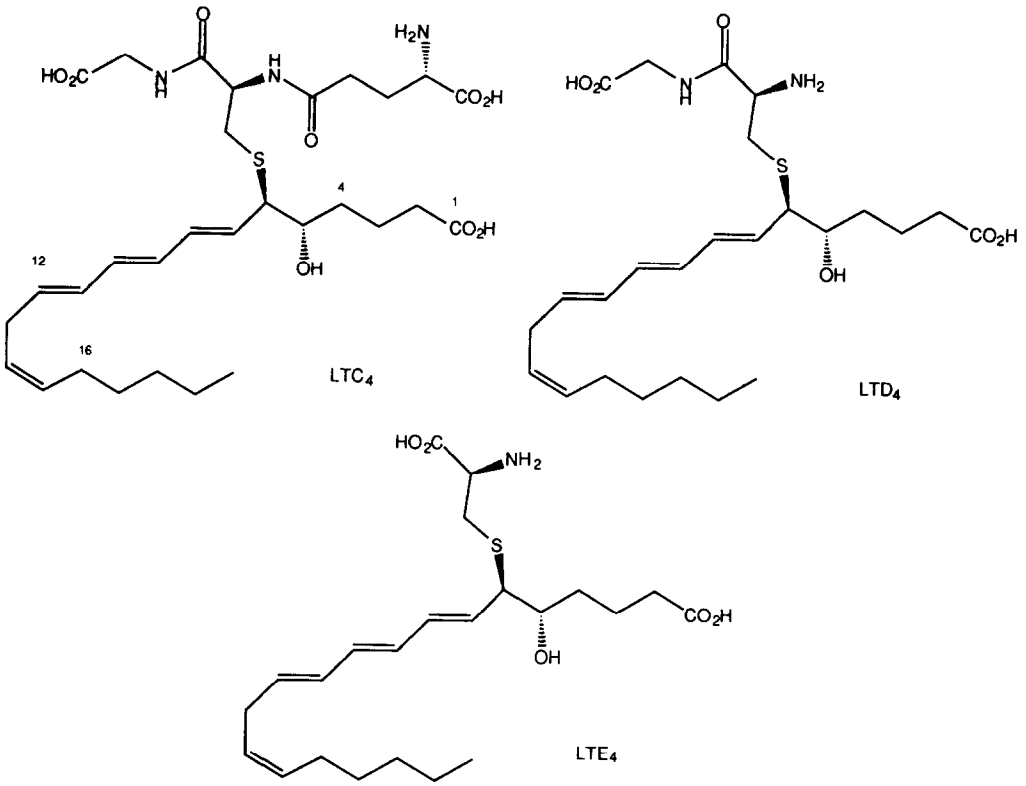

Figure 1. Structures of the cysteinyl leukotrienes.

plored to see if characteristic shapes and dynamic behaviors of the three molecules could be seen and compared.

EXPERIMENTAL SECTION

Molecular dynamics

The molecular dynamics calculations were carried out using AMBER 3.0 running on a Cray-2 computer. The all-atom force field with the 1991 parameter set was used with the addition of the following parameters; bond parameters: CJ-HC 1.08, CJ-CT 1.51; angle parameters: CJ-CJ-CJ 85 120, CT-CJ-CT 85 120, CT-CJ-CJ 85 120, C-CJ-HC 35 120, HC-CJ-HC 35 120, CJ-CJ-HC 35 120, CT-CJ-HC 35 120, C-CJ-CT 70 120, CJ-CT-CT 63 109.5, CJ-CT-HC 35 109.5, CJ-CT-S 50 114.7, CJ-CT-CJ 80 109.5, CJ-CT-OH 50 109.5; torsional parameters: X-CJ-CT-X 6 0 0 3. Periodic boundary conditions were used for the solvent, but all solute–solute nonbonded interactions were calculated and the periodic boundary conditions were not applied to the solute. The shake option was applied to all bonds. An 8-Å cutoff was used for nonbonded interactions. The lowest energy structures of LTC_4, LTD_4, and LTE_4 found in an earlier study[13] were used as arbitrary starting points for the simulations. Gasteiger charges were added and the molecules were placed in 30-Å boxes of water. Each simulation was carried out for 150 ps after heating to 310 K and equilibration. All three simulations departed from their starting conformations during heating and equilibration. Dynamics frames were saved every 0.01 ps during the simulations.

Visualization

The water molecules that were present in the simulations were left out of the visualizations to give a clearer view of the leukotriene molecules. In each graphics frame the planar triene moiety of each leukotriene was always rotated into the (*xz*) plane and centered to make it easier to compare the intramolecular motions of the three leukotrienes. Images were rendered using Wavefront Technologies software on a Silicon Graphics 4D 240GTX graphics workstation. The Color Plates shown in this article are stills from a videotape of the animated visualization. Copies of the videotape (NTSC VHS) will be provided to investigators who request it. The distances between four indicator atoms—the carbonyl oxygen of cysteine, C-16 in the lipid tail, C-12 in the triene moiety, and C-4 in the alkyl acid chain—were calculated. These four atoms were chosen because (1) they are present in all three of the leukotriene molecules and (2) they indicate the relative positions of the four substructures contained by all three leukotrienes (the position of cysteine C=O indicates the position of the amino acid side chain, the position of C-16 indicates the position of the saturated lipid tail, the position of C-12 indicates the position of the triene unit [because the relative positions of the atoms of the triene system are essentially constant, any atom of the triene system could have been chosen], and the position of C-4 indicates the position of the C-1–C-5 chain). Structure–activity studies on the cysteinyl leukotrienes have shown that all four of these substructures contribute to the biological activity of the molecules.[14] To create the ghost images every 100th saved dynamics frame was used, and the atoms

were rendered as opaque spheres with radii 1/20th of the van der Waals radii to produce the overall effect of transparency (70 746 atoms were rendered in each ghost image [Color Plates 3 and 4]).

Digital filtering

Osguthorpe and Dauber-Osguthorpe[11] have demonstrated that low-frequency collective motions can be used to recognize specific patterns of dynamic behavior in molecules. We have developed a filtering algorithm[15] to extract these low-frequency motions from our dynamics calculations. Assuming that the initial digital signal S is an algebraic sum of the low-frequency component U and normally distributed high-frequency noise N, the algorithm extracts U with maximal shrinkage of the data by eliminating as much as possible of N. The digital filtering program was written in FORTRAN-77 and the calculations were carried out on a Cray-2 computer.

Dynamic analysis

Because only the internal motions of each molecule were of interest, the dynamics output was converted to intramolecular distances. The distances between the four indicator atoms were calculated to give a 4 × 4 matrix for each compound at each time point. The filtering procedure described in the previous section was used to remove low-amplitude, high-frequency components of the signal. The signal removed was shown to have the normal distribution of frequencies expected for Gaussian noise. The resulting low-noise signal for each molecule could be represented by 1 of every 110 time points, therefore the number of dynamics frames necessary to describe the trajectory of each molecule was reduced 110-fold by this procedure. For each dynamics frame after filtration the average distance of the four key atoms from their geometric center was calculated and plotted against time to illustrate the dynamic behavior of each molecule (Figure 2).

RESULTS

The results of the dynamics runs were initially visualized as animated ball-and-stick models with shadows projected on the *xy*, *xz*, and *yz* planes (Color Plates 1 and 2). These shadow images clearly show the shape of each molecule in each frame. The lipid tails of all three leukotrienes moved through a wide variety of conformations during the simulations. The cysteinyl arm and the alkyl acid (C-1–C-5) arms were much more mobile in LTE_4 than in LTD_4 or LTC_4. These two arms moved through a wide range of conformations during the LTE_4 simulation and a conformational preference for LTE_4 was not obvious. In LTD_4 the cysteinyl and alkyl acid arms still moved through a wide range of conformations, but in many frames the triene, the cysteinyl arm, and the alkyl arm of LTD_4 form a T shape (in which the triene forms the stem of the T and the cysteinyl and alkyl acid arms form the cross-piece) when viewed in the *xy* plane (Color Plate 1). In LTC_4 the cysteinyl and alkyl acid arms also moved through a wide range of conformations, but in many frames the triene, the cysteinyl arm, its glutamyl arm, and the alkyl acid arm of LTC_4 formed a cup shape (in which the triene forms the stem of the cup and the cysteinyl, glutamyl, and alkyl acid arms form the bowl of the cup) when viewed in the *xy* plane (Color Plate 2).

To allow data from many time points from all three simulations to be seen in the same image along with all of the corresponding molecular structures, we created ghost images. In the ghost images the position of each atom in each frame rendered was represented by a small sphere. The brightness of the image was adjusted so that single spheres were barely visible. Each molecule appears as a cloud that is brighter where many spheres overlap. Color coding the spheres by atom type allows the range of movement of each atom to be seen (Color Plate 3). When the ghost images were color coded by molecule the three ghost images could be superimposed (Color Plate 4).

The average distances of the four selected atoms for each molecule from the geometric center at each time point remaining after filtration were calculated and plotted against time in Figure 2. This plot shows the characteristic frequencies and amplitudes of the breathing motions of the molecules, and shows that their dynamic behaviors are markedly different.

DISCUSSION

The ghost images succeed in conveying much of the essential information from all three simulations in a single image (Color Plate 4). They allow the results of all three simulations to be viewed for all of the heavy atoms in a single three-dimensional image. In this image 70 746 atoms are rendered in each video frame, visually communicating information contained in several gigabytes of numerical output. The T-shaped conformation of LTD_4 and the cup-shaped conformation of LTC_4 show up clearly. A small number of conformations of LTC_4 that overlap the T-shaped conformation of LTD_4 can be seen, and among the widely scattered conformations of LTE_4 some can be seen that overlap LTD_4 and LTC_4 conformations.

Leukotriene E_4 conformations are scattered throughout and in between the regions of conformation space that contain LTC_4 and LTD_4 conformations. Leukotriene C_4 conformations are concentrated in the cup-shaped conformations, although some spillover into T-shaped conformers can be seen in the ghost images. Leukotriene D_4 is concentrated in the T-shaped conformation space. This picture is consistent with what is known about cysteinyl leukotriene receptors in various species.[16] In guinea pig, the experimental animal most often used to develop cysteinyl leukotriene receptor antagonists, the available evidence suggests that at least three cysteinyl leukotriene receptors are present: an LTC_4 receptor and two LTD_4 receptors. Leukotriene E_4 appears to bind most strongly to one of the LTD_4 receptors. The guinea pig lung LTC_4 receptor might recognize an abundant cup-shaped conformation of LTC_4 that is not occupied by LTD_4 or LTE_4, and the guinea pig lung LTD_4 receptor might recognize an abundant T-shaped conformation of LTD_4 that is not occupied by LTC_4 but that is occupied to some degree by LTE_4. In human bronchus, on the other

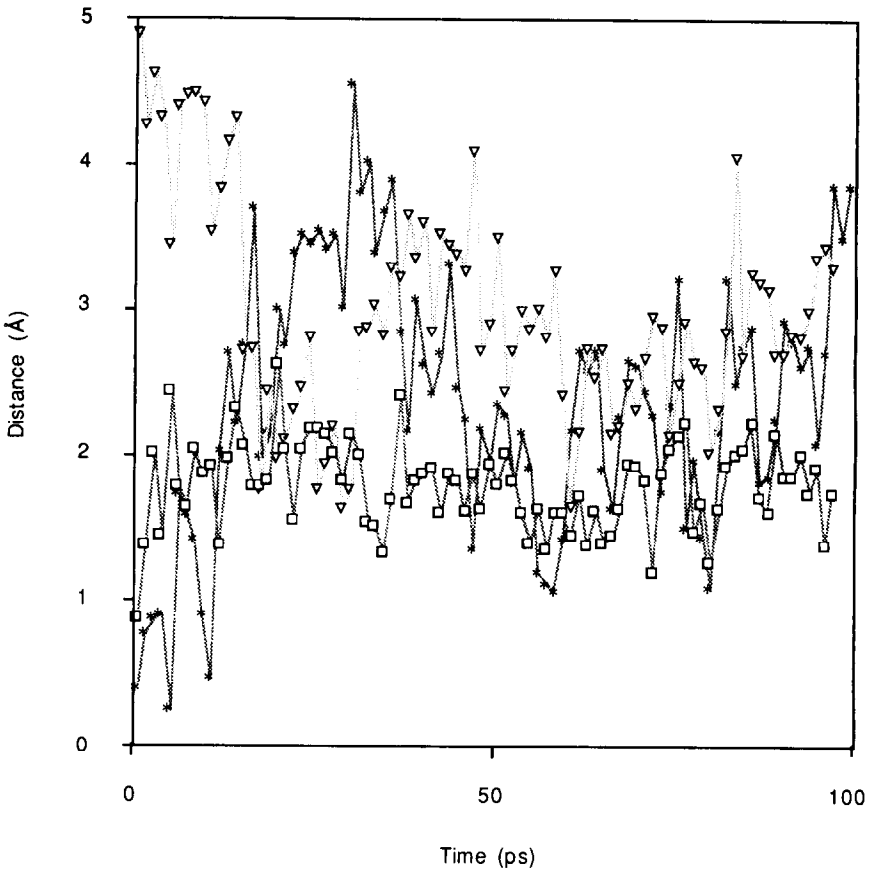

Figure 2. Average distance of the four key atoms from their geometric centers plotted against time. (□) LTC_4; (△) LTD_4; (*) LTE_4.

hand, all three cysteinyl leukotrienes appear to act at one type of receptor. In this case one of the more peripheral LTC_4 conformations which can be occupied by LTD_4 and LTE_4 as well, may be recognized by the receptor. While such conformations of LTC_4 were relatively uncommon in this simulation, they may in fact be abundant conformations that would have been sampled more thoroughly if the simulations had been longer.

The plots of the internal geometries of key atoms of each molecule against time (Figure 2) show that LTC_4, LTD_4, and LTE_4 have characteristic breathing motions, and it is interesting to speculate that the characteristic motions of the molecules, in addition to their characteristic shapes, charge distributions, and hydrogen-bonding properties, might play a role in the ability of receptors to discriminate among these ligands.

The cup shape observed for LTC_4 suggested to us that the three carboxylic acid groups of LTC_4 might be able to chelate calcium. Indeed, preliminary results of free energy perturbation studies suggest that LTC_4 may be able to form a stable Ca^{2+} complex at physiological concentrations of Ca^{2+}.[17] Thus both free and Ca^{2+}-complexed cup-shaped conformations of LTC_4 may be considered as models for receptor-binding conformations of LTC_4. Calcium ion chelation by LTC_4 might also explain ionophoric properties that have been reported for LTC_4 in some biological systems.[13,18]

The T-shaped conformation of LTD_4, which this work suggests to be at least one of the preferred conformations of LTD_4 is completely consistent with the results of NMR studies[4] of LTD_4. These studies had suggested that *trans* conformations of the protons on C-6 and C-7, of the sulfur atom and C-4, and around the C-8–C-9 and C-10–C-11 bonds were preferred. All of these relationships are found in the T conformation of LTD_4 (Color Plate 1).

The observation[16] that the most potent LTD_4 receptor antagonists contain a linkage designed to twist the acid chains out of plane relative to the triene equivalent also suggests that T conformations of LTD_4 may be relevant. Gilmore and Todd used the T-shaped conformation of LTD_4 as a guide in designing a new series of extremely potent LTD_4 receptor antagonists.[19]

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