Intermolecular enzyme–ligand animation in the active site of porcine pancreatic elastase with acetyl-alanine-proline-alanine by means of molecular dynamics calculation

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An interactive molecular display system has been developed to enable the visualization of the 540 conformations derived from a molecular dynamics calculation of the fluctuations of the enzyme–ligand complex formed between porcine pancreatic elastase (PPE) and acetyl-alanine-proline-alanine (APA). Dynamical interactions between the receptor and the inhibitor are observed at the active site, e.g. the pyrrolidone ring of the ligand 2-proline residue is observed to flex via restrained dihedral angle rotations; the terminal acetate moiety is seen to move between two adjacent binding loci. An animated molecular graphics display, linked to a molecular or stochastic dynamics method, is an instructive and predictive tool for investigating dynamical interactions of enzyme–ligand binding.

Keywords: animated molecular graphics, molecular dynamics, inhibitor design, porcine pancreatic elastase (PPE), molecular modelling, force-field calculation

Received 21 April 1986
Accepted 13 May 1986

While the computational demands of macromolecular dynamics calculations are widely recognized, one really has to undertake such a calculation in order to experience the overwhelming amount of output. While a parameter measuring stability or change may be expressed numerically, information of chemical/structural significance may be lost if a graphical tool is not available for following the discrete steps in the calculation. The authors therefore report several aspects of the interactive graphics display, in stereo, of a 31.5 ps dynamics calculation.

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The molecular dynamics calculation of a protein molecule was initiated by Karplus and McCammon[1,2] as a means of studying the time-dependent fluctuations of macromolecules. Furthermore, the molecular dynamics method is finding increasing utilization in computer-assisted drug design. However, the analytical tools used by these programs cannot go far enough to give a global view of the fluctuating structure; an interactive graphical display is clearly essential for the evaluation and presentation of such extensive calculations. In particular, when studying drug–receptor interactions, graphical output is indispensable in order to observe the overall fluctuations of individual atoms and groups of atoms. In studies using macromolecular dynamics, the fluctuations of the binding substrate and its receptor can be observed. The authors have developed several programs for molecular graphics[3,4] and applied them to the task of molecular modelling[5]. A program called Pix[4] has been modified to permit the interactive display of multiple frames, as a type of molecular 'animation'; this has resulted in a program called Kino, which is reported here.

The field of molecular graphics has made enormous progress in both hardware and software for the display of molecular structures. The quality and clarity of vector and raster displays continues to improve, even as the size of molecules held in databases increases. Progress in other areas, such as molecular dynamics and force-field calculations, allows hundreds of conformations to be entered into databases as a single entry. Many excellent graphics packages would be severely challenged if an investigator wanted to view many consecutive conformations interactively.

The viewing of consecutive conformations generated by molecular dynamics calculations has been called 'animation' and has been graphically represented by single frame motion picture photography for off-line viewing[1] or by special arrangements of mini and/or mainframe computer-graphics links[6,7]. An elaborate solution

to the problem has been outlined by Todd and Gillet[6] in their description of the Winchester Graphics System. The method presented here uses a VAX minicomputer, vector display, and off-line, x–y plotter.

Experimentally, porcine pancreatic elastase has been found[8] to bind two tripeptide (acetyl-Ala-Pro-Ala) ligands under equilibrium conditions. The authors wanted to model these two ligation loci and probe their binding modes by means of dynamical calculations, the results of which will be presented elsewhere. The evaluation of the large amount of output demanded the use of a graphical tool. The 1.65Å resolution, refined atomic coordinates served both as a starting point and a structural reference for these calculations.

The dimensions of the problem are straightforward; the 3D coordinates of each 'frame' of a molecule containing $\approx$2000 atoms must be presentable at a desired rate of $\approx$8–24 frames/s. The user who is accustomed to the interactive tools common to current systems (zooming, pointing, etc.) expects to be able to use these tools, and requires them in order to derive meaningful information.

In order to optimize a structure and quantify the results, molecular modelling is generally accompanied by a molecular mechanics calculation[9] which presents a static relationship between receptor and ligand (drug) molecules. Various assumptions are built into any such modelling hypothesis; while molecular mechanics calculations are designed to seek out the minimum energy conformation of any such complex, they can say nothing about other, neighbouring minima because of the energy barriers separating them. The perturbations introduced as part of molecular dynamics calculations make it possible to jump local barriers in order to seek other, hopefully lower, minimum energy conformations.

When considering the importance of rational drug design, especially in the design of enzyme inhibitors, these dynamical interactions may add essential information. Conversely, without it, the chemist is rendered 'blind' and his intuitive sense must be put aside. There are a few instances where such graphics have been committed to motion picture film to be shown to large meetings, but it is important that this graphical tool be available to the individual investigator for his own calculations. The phrase 'a picture is worth ten thousand words' gives a rough proportionality to the data compression powers of interactive graphics, as matched by the image processing power of the creative chemist.

Some mechanistic details of Kino will be discussed and the graphical analysis of the molecular dynamics results also described. These results may, by extrapolation, be extended to a number of other enzymological and pharmacological systems.

METHOD

A molecular dynamics calculation has been performed to investigate the complex formed between porcine pancreatic elastase (PPE) and acetyl-ala-pro-ala (APA) in the range 0–31.5 ps using a modified Gromos package[10]. This molecular modelling simulation[11] is performed by means of force-field calculations, based upon the X-ray crystal structure[8]. After an equilibration stage of 4.5 ps, coordinates are stored after every 0.05 ps simulation. From the whole dynamics calculation, 540 conformations are obtained. A new graphics display program Kino will be reported here (see Figure 1); it uses the

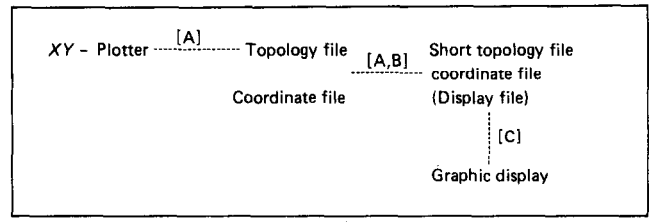

Figure 1. Overall system for the molecular animation and programs. Programs used are: the modified Gromos package extended to molecular graphics developed in this study; B: Flicks; C: Kino

Vector General 3-DM display, controlled by a VAX 11/750. Initially, the structural output is stored as ASCII files. For efficiency, the data is converted to compressed, binary files. This binary data is then converted to direct access display files using the program Flicks and stored on disc for rapid access during the display process.

The Vector General used in these studies is an early model whose display buffer resides in the main memory of the VAX. At a drawing rate of about 15 μs/short vector (6 byte), the transfer of graphical information to the display takes from a quarter to a half of the I/O bandwidth. Individual conformations are transferred from disc to memory as single records from a direct access file: 24 frames/s of 2000 vectors each take only one quarter of the I/O bandwidth. As discussed later, the usual display mode is a 'scoop' of perhaps one fifth of this size. Satisfactory performance is obtained while running as an ordinary interactive process (with no special priority), sharing the CPU with several other users and batch jobs.

Both for computational efficiency and reducing the visual overload of the viewer, the user may select a subvolume of the molecular model for detailed investigation. Connectivity information about the 'scooped' region is stored in an invariant topology file. After several trials, a 12 Å scoop was found to be a reasonable compromise between detail and complexity. the centre of the scoop is the Pro Cα atom of the ligand oligopeptide. Although the whole structure is used in the calculation, only atoms within this radius in the starting structure are consistently extracted and placed in the corresponding condensed coordinate files.

First, for simplicity, hydrogen atoms are removed; then, a short topology file is made in the scooped region. Bond connection data and the coordinates of each coordinate file are generated for Kino, which creates animated views continuously, under user control (see Figure 2). The program has been written to display 'frames' at a variable speed (in forward or reverse order), with a frame freeze option. Typical maximum speeds are 8–24 frames/s, depending upon the number of vectors/frame. This speed is satisfactory for watching concerted conformational changes in stereo. Considering the complexity of even a scooped region of a protein, stereo viewing is absolutely essential to comprehend the subtle structural shifts being presented; a 'rocking' picture (kinetic depth effect) only adds another perturbation to a monoview. Additional features, partially implemented, permit interactive queries of atom residues and types, atom contact distances and other geometric features. This data will be used in structural analysis, for example, bond distances, or root mean square fluctuations of specific bonds and dihedral angles.

Pictures with hydrogen atoms included can also be displayed, but those without hydrogen atoms have been found to be visually preferable. The pictures with hydrogen atoms are effective in the narrow scoop-region for the analysis of hydrogen bonding. The viewpoint options, including pan, rotate, zoom and stereo, are under full operator control. The viewer can select a discrete atom using a unique pointer. Thus, the fluctuations of individual atoms can be observed on a graphic display, together with the residue name, number, and atom name, all of which are derived from the topology file. This application is important in studying the atom or residue fluctuations in active site. The number of each frame is displayed; thus, individual frames may be selected for offline plotting on an HP-7550 plotter (see Figures 3 and 4). Residue labels are included in the plots as an output option.

It it possible for the user to observe that some groups fluctuate somewhat more than others, especially those external residues not committed to surface H-bonding. Especially striking is the observation of the vibrational modes of the ligand 2-proline pyrrolidone ring; each of the nonchain Cβ, Cγ and Cδ ring atoms is involved in motions above or below the plane of the ring. A 2:3 split is rarely seen; this situation approaches a 'boat'-like conformation. Other ring structures in the receptor region are likewise seen to deviate temporarily from their normal, planar conformation.

The biggest structural change, and also the enzymologically most significant, is a partitioning of the acetate terminus of APA between a 'comfortable' association (H-bonding) with the Ser 195 Oγ on the surface of the receptor and a burrowing of the acetate methyl group into the S_1 specificity pocket (with a related H-bonding association of His-57). Without graphics, such concerted motions would have to be identified from the coordinates. This is a difficult task because they would have been made obscure by the abundance of the many outputted structures.

By changing the topology file, several types of pictures (e.g. mainchain with or without side chain) can be drawn using application programs. All programs used in this system are written in FORTRAN 77 and this animated graphic system will soon be interfaced to other molecular dynamics packages such as Amber[9].

```
Run KINO                                                    (1)
Move - Draw - Name - File:  MDAPAATOM.MDD (topology file)   (2)
PPE - APA System                                            (3)
Direct Access Display File:  DIRECTAPA.DAT (data file)      (4)
Number of Configurations : 540
(5)

NCONFG      1
NCONFG      2
NCONFG      3
NCONFG      4
            .
            .
            .
NCONFG    540
NCONFG      1
NCONFG      2
            .
            .
            .
(circular addressing)
```

Figure 2. Access of the molecular animation program Kino. Each step is: (1) program start, (2) short topology input, (3) job name output, (4) coordinate file input, (5) total number of configurations input. In molecular animation, the number of the drawn configuration is simultaneously displayed, as 'NCONFG 1'

RESULTS AND DISCUSSION

An excess of substrate was added to a PPE crystal, in the presence of buffer. The substrate was cleaved and the tripeptide product molecules complexed. The single crystal structure[8] of the enzyme–ligand complex between PPE and APA was refined to 1.65Å resolution; two molecules of the ligand are bound backward in the extended binding site, indicating both the breadth and, in particular, the partially understood specificity of binding. Another binding study, using X-ray and NMR spectroscopy[11], showed similar, anomalous binding. Here, because the substrate must bind prior to proteolysis, a prediction and study of the 1:1 complex would be useful for the design of more potent inhibitors and a study of the inhibition mechanisms. Because of the relationship of the elastases to degenerative diseases (pancreatitis, emphysema, arthritis), there is considerable interest in such modelling and structural studies.

The parameterization and computational details of the dynamics calculation will be reported elsewhere. It is useful to recall that several dynamics calculations have been reported, and that only one graphical solution to the problem of visualization of such a mass of data has been presented[6], using specialized hardware. This method will be particularly useful when it is implemented on standard hardware and software (VAX, PS330).

After the files are prepared, it takes a few seconds to give the necessary information to start the display of the data. Picture scale, stereo option, picture intensity, etc. are set initially. The display rate (forwards, freeze, backwards) is set by adjusting a control dial. For an observer acquainted with the extended binding site of PPE, hydrogen bonds may be visualized intuitively. Whether the two principal binding modes of the acetate moiety (see Figures 3 and 4) could have been derived from the masses of data without graphics assistance is open to question. In the present study, this graphics tool makes the visualization of various binding modes manifestly clear, suggesting possibilities for drug or inhibitor design that would have been far less apparent from static studies.

The active site region of PPE is extended but the size of APA is comparatively small. During the time range 0–31.5 ps, the ligand was expected to move around the active site, because in the crystal structure the second substrate molecule binds to an adjacent area of the active site. On a graphic display, the substrate was observed not to move widely in its position. This was probably because the ligand binds strongly to the active site atoms in this locus by directed intermolecular forces and the competitive role of water was, in general, not included in the calculations.

The observation of the results of consecutive calculation has enabled the visualization of the actual enzyme–ligand interactions. In each picture, derived structural parameters may be extracted by Kino and the atoms which have to be labelled interactively are highlighted using an arrow. Stereo viewing helps to show the 3D complexity of the structures. Because of the considerable structural detail still in the scoop region and

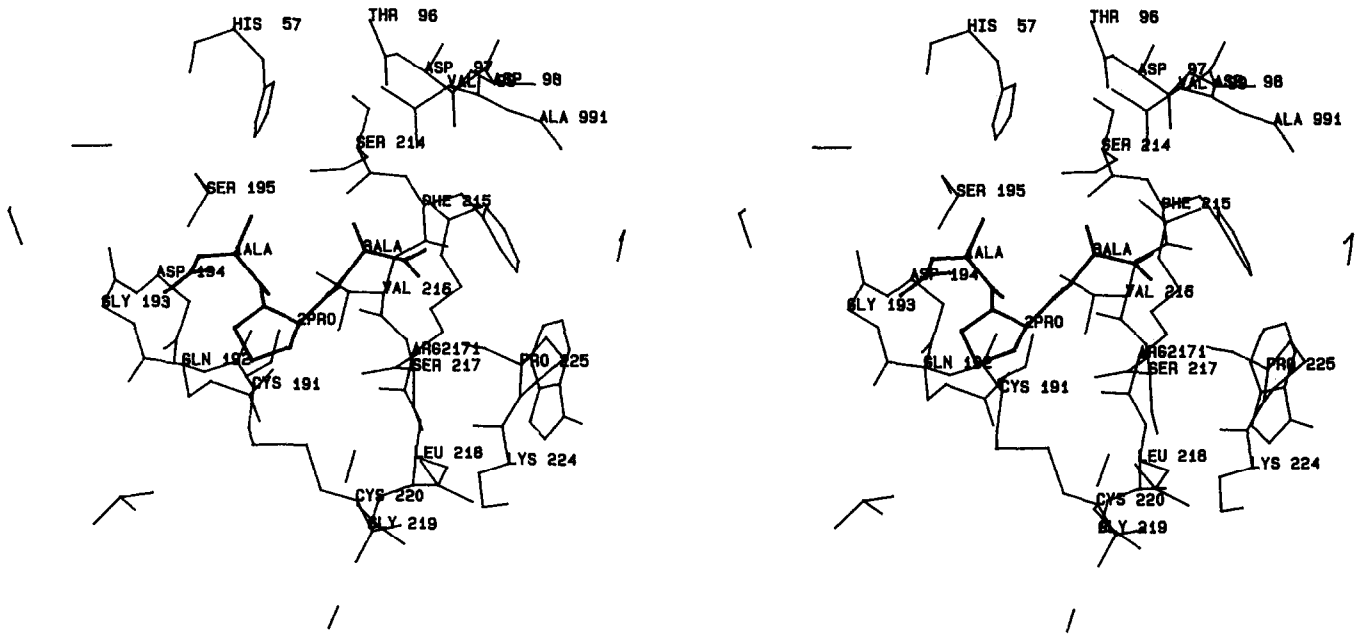

Figure 3. 12 Å scoop region of Cα atom of the 2-proline residue of APA1 is shown. Each residue of the substrate is denoted by 1Ala, 2Pro, and 3Ala and the substrate is located at the centre of the Figure

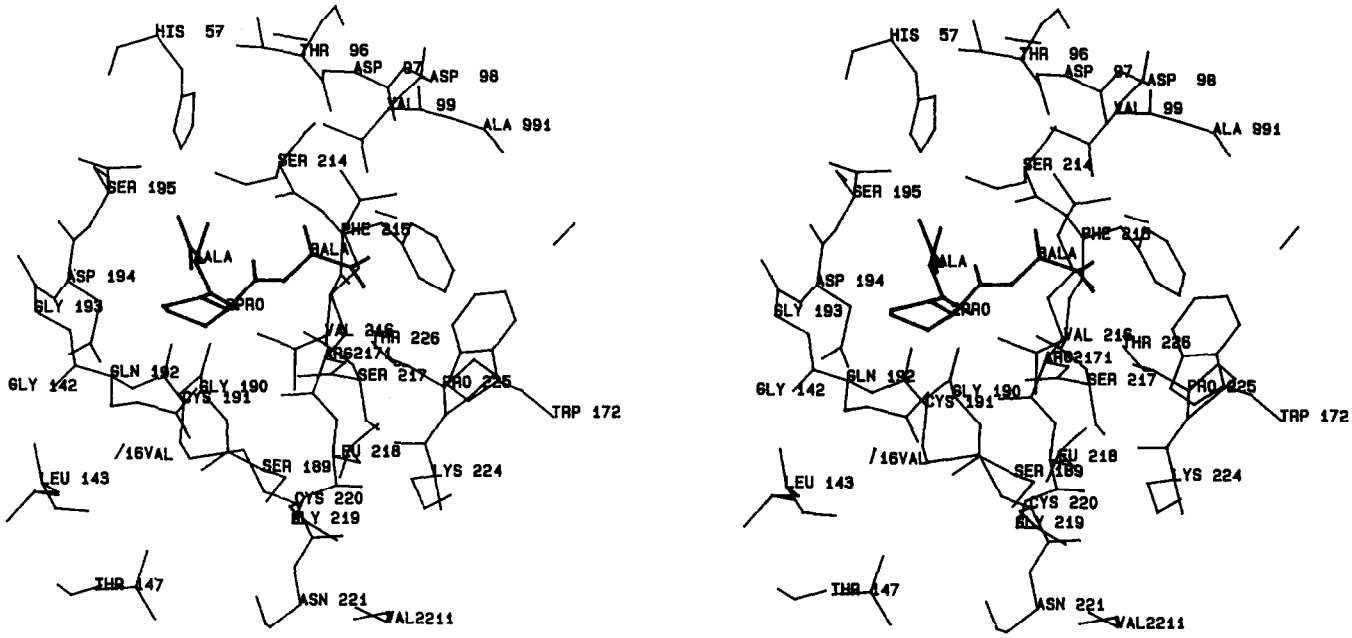

Figure 4. Changed conformation from that shown in Figure 3. The pyrrolidone ring of the 2-proline residue has been bent; the acetyl group is 'out'

the cumulative importance of subtle interactions, when H-bonding and van der Waals forces dominate binding affinities, the detailed evaluation of individual and consecutive frames, in stereo, is important for the analysis of the molecular dynamics data. This is because the dynamics aspect of molecular structures is very significant in enzyme–substrate binding studies.

CONCLUSION

An animated molecular graphics method with a Vector General display controlled by a VAX 11/750 has been developed and the fluctuations of 540 conformations fully observed. Although the side chain atoms exhibit wide fluctuations, the global enzyme structure does not change appreciably. In addition to the observation of the enzyme structure, the fluctuations of the ligand are considered. As a result, the pyrrolidone ring of the 2-proline residue undergoes various conformational distortions; this observation has been captured by an animated graphic method. For more precise analysis, some statistical calculations using the coordinates of these visually selected conformations may be employed. Consequently, it is emphasized that the animated depiction of all conformations can greatly help subsequent statistical analysis and support it by directing the investigator to highly relevant frame sequences.

ACKNOWLEDGEMENT

The authors are thankful to Dr R Radhakrishnan for helpful discussion of the X-ray crystal structure and for

providing and modifying HP plotting programs. Thanks are specially owed to Prof J A McCammon, University of Houston, for his initial suggestions for this work. Support has been provided by the Vice-Provost for Computing, Texas A&M University; the Office of Naval Research; the Robert A Welch Foundation and the Texas Agricultural Experiment Station.

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