ELSEVIER

Computer Physics Communications 91 (1995) 263–273

Computer Physics Communications

A method to explore transition paths in macromolecules. Applications to hemoglobin and phosphoglycerate kinase

Christophe Guilbert, David Perahia*, Liliane Mouawad

Laboratoire d'Enzymologie Physico-Chimique et Moléculaire, Unité de Recherche du Centre National de la Recherche Scientifique, Université Paris-Sud, 91405 Orsay cedex, France

Received 17 October 1994; revised 23 December 1994

Abstract

A method to find low energy paths in macromolecules is described. It can be applied either to determine paths between two given energy minimum conformations, or to explore low energy paths departing from one energy minimized conformation. The principle of the method consists in carrying out energy minimizations or molecular dynamics simulations with root mean square distance constraints with respect to a reference structure. The method is illustrated by applications to N-methyl-alanyl-acetamide, hemoglobin and phosphoglycerate kinase.

Keywords: Macromolecules; Conformational pathways

1. Introduction

Most large amplitude motions corresponding to a conformational change between two states in biological macromolecules play an important role in their function. Among the numerous examples we can mention the domain motions in kinases in which open forms are necessary for substrate binding or product release, and closed forms for the enzymatic catalysis [1,2]; allosteric motions in hemoglobin transition between states with low and high affinity for ligand binding [3]. These motions involve collective displacements of atoms which are mostly difficult to study by conventional molecular dynamics or Monte Carlo simulations since they correspond in general to time scales extending beyond the nanosecond. Although a normal mode analysis could characterize these movements around a minimum energy conformation, they remain valid only for relatively small displacements, and one has to devise methods to explore pathways far from the given structure.

Various computational methods were developed in order to explore reaction paths in molecules. Efficient algorithms were proposed using modified Newton–Raphson techniques to proceed "uphill" along one eigenvector mode from a minimum on the potential energy surface to a transition state [4,5]. These techniques use only one starting structure on the path, but unfortunately they cannot be applied to macromolecules containing thousands of atoms since the systematic diagonalization of large Hessian matrices required by these methods is prohibitively time consuming and uses a large computer memory.

Other algorithms were developed; they consist in generating an ensemble of conformational points between two energy minima, which are simultaneously

* Corresponding author.

Elsevier Science B.V.
SSDI 0010-4655(95)00052-6

energy minimized under constraints in order to constitute the transition path [6–8]. These methods are global since an entire set of points is considered simultaneously in the search for a low energy path. Originally such a method was developed by Elber and Karplus [6], where the starting set is obtained by a linear interpolation of Cartesian coordinates between the two distant structures; it was applied to determine the reaction path in myoglobin. Ech-Cherif El-Kettani and J. Durup [8] proposed a variant of this method in which the Cartesian coordinates were replaced by internal coordinates in terms of Jacobi vectors to represent the pathway between two crystallographically known structures of Citrate Synthase; this algorithm provided a better initial interpolated pathway to start the computations. Another global method was proposed recently by Fischer and Karplus [9], where the intermediate structures are not only generated along the direction between the two minima, but by bringing the "hot spots" regions along the path down into a nearby valley linking the saddle points. A built-in aspect of all these global methods is that two conformations are needed to establish a reaction pathway. This constitutes a limitation for determining pathways between two states of a macromolecule for which only one experimentally resolved structure exists. Another point which may be raised concerning these methods is that they provide in general a single low energy pathway, the one which is reached first from the initial interpolated set of conformational points between the two end points.

Other approaches for determining energetically more relaxed paths consist of directed molecular dynamics simulations [8, 10]. The principle is to introduce a bias in the molecular dynamics by periodic velocity assignments that drive the system in a desired direction.

In this article we present a new approach which makes use of distance constraints to determine transition paths between distant conformations of macromolecules, and to explore low energy valleys departing from one of them, in order to find other stable structures. The principle of the method of Path Exploration with Distance Constraints (PEDC) is to follow low potential energy valleys by carrying out energy minimizations or molecular dynamics under constraints which either push the starting structure away from the initial point on the path, or pull it toward the final point. The method is presented in detail in the next section, followed by a test on N-methyl-alanyl-acetamide (NMAA). Then it is applied to the macromolecular systems, hemoglobin and phosphoglycerate kinase (PGK).

2. Methodology

The principle of the PEDC method is to explore the potential energy surface by displacing a structure from a minimum energy conformation; the displacement is done by addition of a mass weighted root mean square distance (mrmsd) constraint potential (V_{dist}) to the classical internal potential energy function (V_{int}). This constraint is necessary to displace and maintain the structure at a given mrmsd from a reference; it is gradually modified during energy minimizations or molecular dynamics simulations in order to make the system evolve on the potential energy surface. Two other constraints are added to prevent the displaced structure from overall translation (V_{trans}) and rotation (V_{rot}).

The potential energy function for a displaced structure J is of the form

$$V^J = V^J_{\text{int}} + V^J_{\text{dist}} + V^J_{\text{trans}} + V^J_{\text{rot}}\,, \tag{1}$$

where V^J_{int} is the internal potential energy function of the molecule that is used in standard simulation programs, and V^J_{dist}, V^J_{trans} and V^J_{rot} are the constraint potentials. The distance constraint is

$$V^J_{\text{dist}} = \tfrac{1}{2} k_{\text{dist}} \left(d^J_R - d^J_0\right)^2\,, \tag{2}$$

d^J_R being the mrmsd between the structure J and the reference structure R,

$$d^J_R = \sqrt{\frac{\sum_{i=1}^{N}\sum_{\alpha=1}^{3} m_i \left(x^J_{i\alpha} - x^R_{i\alpha}\right)^2}{M}}\,, \tag{3}$$

where m_i is the mass of atom i, $x^J_{i\alpha}$ and $x^R_{i\alpha}$ are the coordinates of atom i in structure J and R, respectively, α is an index that corresponds to the Cartesian coordinates of atom i, M is the total mass of the molecule, and N the total number of atoms. d^J_0 is the desired mrmsd between structures J and R; the value of d^J_0 is changed for the next structure $J+1$. The overall translation constraint potential is

$$V^J_{\text{trans}} = \tfrac{1}{2} k_{\text{trans}} \left|\boldsymbol{r}^J_{\text{CM}} - \boldsymbol{r}^R_{\text{CM}}\right|^2\,, \tag{4}$$

where r^J_{CM} and r^R_{CM} are the Cartesian coordinate vectors of the center of mass of structures J and R, respectively.

The overall rotation constraint potential is

$$V^J_{rot} = \tfrac{1}{2} k_{rot} \left| \sum_{i=1}^{N} m_i \left(r^R_i \times \left(r^J_i - r^R_i \right) \right) \right|^2 . \qquad (5)$$

r^J_i and r^R_i are the Cartesian coordinate vectors of atom i with respect to the center of mass of structures J and R. k_{dist}, k_{trans} and k_{rot} are the force constants for the three types of constraints.

With molecular dynamics simulations at 300 K, it was found more appropriate (see discussion in the application section) to consider the following distance constraint potentials:

$$\begin{aligned} V^J_{dist} &= \tfrac{1}{2} k_{dist} \left(d^J_R - d^J_0 \right)^2 , \quad \text{if } d^J_R < d^J_0 , \\ V^J_{dist} &= 0 , \quad \text{if } d^J_R \geq d^J_0 , \end{aligned} \qquad (6)$$

in the case where the initial point is the reference, and

$$\begin{aligned} V^J_{dist} &= \tfrac{1}{2} k_{dist} \left(d^J_R - d^J_0 \right)^2 , \quad \text{if } d^J_R > d^J_0 , \\ V^J_{dist} &= 0 , \quad \text{if } d^J_R \leq d^J_0 , \end{aligned} \qquad (7)$$

in the case where the final point is the reference. These potentials allow the system to move forward from the initial point, and forbit it from going backward, with the advantage to have simulations without any constraint when the system evolves in the desired direction.

There are different ways to choose the reference structure, to initiate the displacement, and to carry out the computations.

2.1. Choice of the reference structure

(1) When only one conformation of the protein is known, one has to use it as a reference structure, therefore the d^J_0 value must be increased gradually to draw the system away from the reference, which is also the initial point on the path. (2) On the other hand, when two conformations are known, the final point on the path may be chosen as the reference structure, thus d^J_0 should be decreased.

2.2. Initiation of the displacement

When the starting point on the path coincides with the reference structure, at the first step of the simulation, the gradient of the distance constraint potential, V_{dist}, is indeterminate. Thus the starting structure must be slightly displaced (e.g. 10^{-4} Å) in a random direction; the energy minimization under distance constraints will drive the system along the direction of the lowest energy valley. But one useful and practical way to initiate the displacement is to use the direction of a normal mode that contributes the most to a movement of biological interest [11].

2.3. Algorithms

2.3.1. Determination of the pathway with energy minimizations

The algorithm is presented in Fig. 1. The starting structure is first energy minimized to constitute the first point of the path. The force constants for the constraint potentials (Eqs. (1)–(5)), k_{dist}, k_{trans} and k_{rot}, are then set up. Two cases were considered in the algorithm. On the one hand, when the reference structure coincides with the starting structure (case A, in bold in Fig. 1), d^J_0 (see Eq. (2)) is increased by Δd from 0 to d^M_0, which is the maximum mrmsd to which one wishes to explore the potential energy surface. On the other hand, when the reference structure is the final point (case B, in italics in Fig. 1), d^J_0 is decreased by Δd from d^M_0 to zero; d^M_0 is in this case equal to the mrmsd between the final and initial points. Δd is the mrmsd between the successive displaced structures $(J, J+1)$; it should be sufficiently small (e.g. 0.1 Å) to provide a precise description of the pathway.

In case A, in the first move, the structure is slightly displaced along a given direction. Then in both cases A and B, at each iteration step, the d^J_0 is updated, which modifies the constraint potential energy (Eq. (2)), and causes a displacement during the next energy minimization.

2.3.2. Determination of the pathway with molecular dynamics

When the reference structure is the initial point (case A), the structure is displaced by energy minimizations to an mrmsd equal to the observed root mean square fluctuations of atoms at the temperature

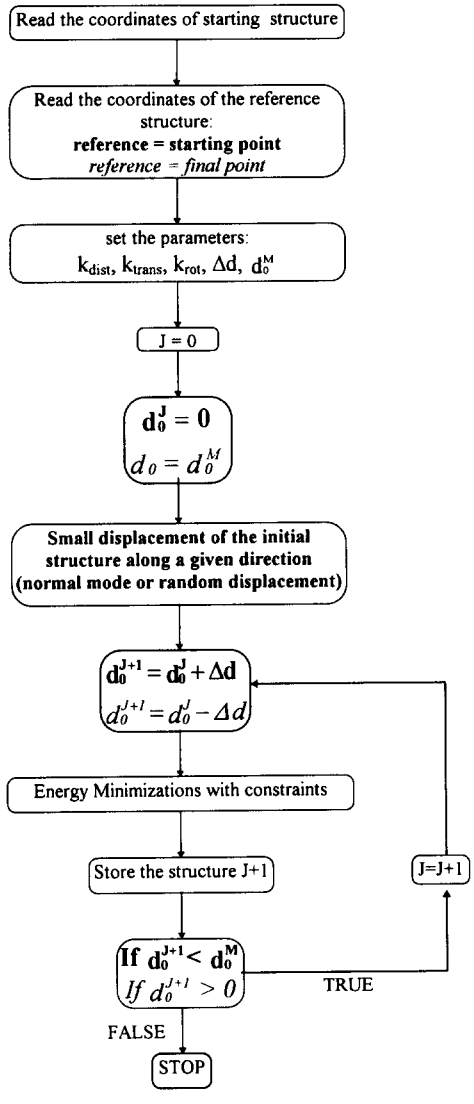

Fig. 1. Algorithm used in the PEDC method. Sections written in bold concern the conformational exploration taking the first structure as reference; those in italic concern conformational exploration taking the last structure as reference.

considered. Then the heating in molecular dynamics is carried out under the distance constraints presented in Eq. (6), with a constant value for d_0^J. The molecular dynamics simulations subject to constraints are continued by maintaining the temperature constant by periodic velocity scaling; d_0^J is increased gradually as indicated in the algorithm for minimizations in Fig. 1, and the coordinates are stored at fixed time intervals.

When the reference is the final point (case B), the procedure is the same as described above, but the distance constraint potential is replaced by that defined in Eq. (7) and d_0^J value is decreased during the computations.

3. Applications and discussions

To illustrate the PEDC method, it was first applied to a small molecule, N-methyl-alanyl-acetamide (NMAA), and then to the macromolecules hemoglobin and phosphoglycerate kinase (PGK). In the former case, we examine the transition paths between minimum energy conformers by applying variants of the PEDC method. In the case of hemoglobin, the PEDC method with constrained energy minimizations was used to determine the transition path between the T- and R-states of this molecule. A third illustrative application consisted in using the PEDC method with molecular dynamics simulations at 300 K in order to obtain a closed (catalytic) form of PGK from its crystallographically known open structure. The calculations were performed with CHARMM23 [12] in which we implemented our algorithms.

3.1. N-methyl-alanyl-acetamide

The advantage of using N-methyl-alanyl-acetamide is that its conformational space is described essentially by two degrees of freedom, the dihedral angles ϕ and ψ around the central α-carbon atom (Fig. 2). Thus it is easy to evaluate the paths obtained by our algorithms by representing them on the detailed (ϕ,ψ) adiabatic energy map. This map was constructed by minimizing the energy of the molecule where pairs of (ϕ,ψ) dihedral angles were constrained to have values on a grid. Calculations were carried out using the parameter set 19 of the CHARMM program [12]. The paths obtained between two given energy minima on the adiabatic map are presented in Fig. 3a.

In the first test the reference was chosen to be the point A, and the first move was done along the lowest frequency mode vector pointing to B, and in the second test the reference point was B. In both tests the starting point was A. The force constants for the constraint potentials were k_{dist} = 1000 kcal/mol/Å^2, k_{trans} = 100 kcal/mol/Å^2, k_{rot} = 0.001 kcal/mol/Å^4 a.u. and Δd = 0.05 Å. Since the mrmsd between points A and B is

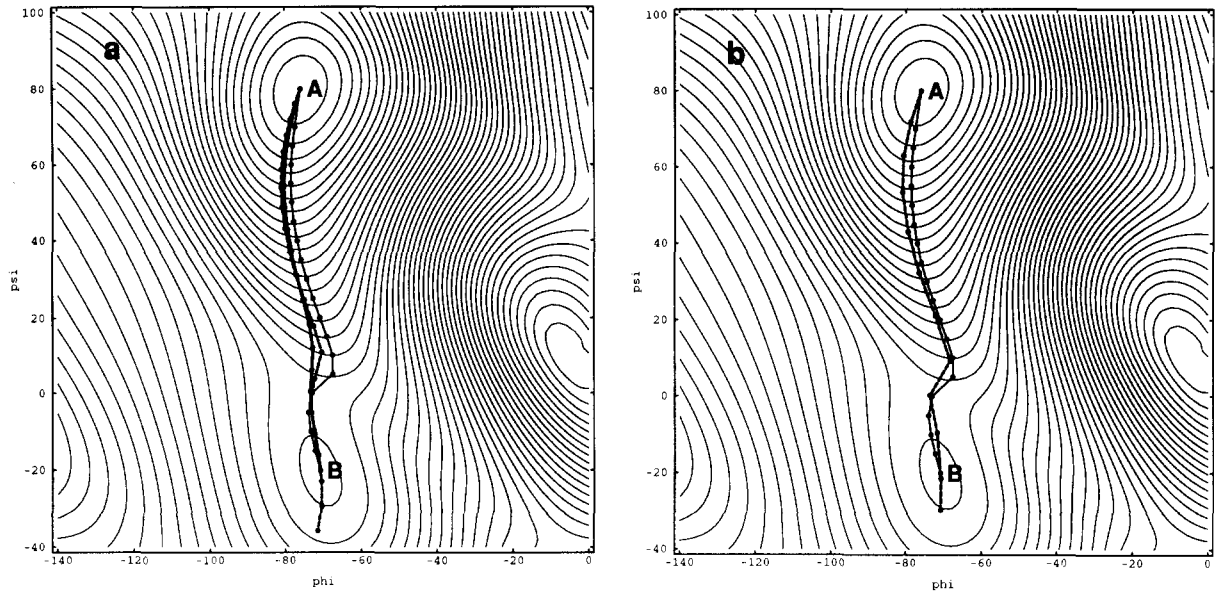

Fig. 3. (ϕ,ψ) adiabatic energy contour plots of N-methyl-alanyl-acetamide containing the two energy minima A and B. (a) Thin solid line: the lowest energy valley between A and B determined by examining the grid values; thick solid line: path determined with PEDC method taking point A as reference; dashed line: path determined with PEDC method taking point B as reference. The contour lines represent energy differences of 0.2 kcal/mol; (b) solid line: paths based on grid values; dashed line: path obtained with the mobile reference algorithm.

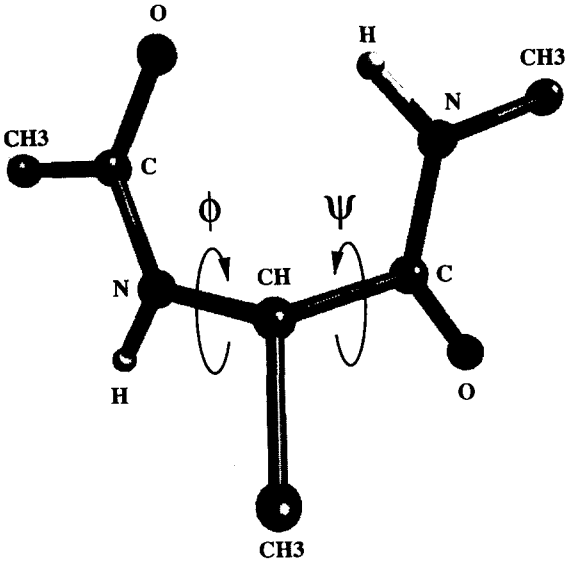

Fig. 2. Structure of N-methyl-alanyl-acetamide.

equal to 0.87 Å, in the first test d_0^J was increased from 0 to 0.87 Å, while in the second test it was decreased from 0.87 Å to 0. Both trajectories are reported on the map (Fig. 3a); they show that the two paths determined either by pushing the structure from A or pulling it toward B are close to the lowest energy path. The latter was obtained by minimizing the energy of the molecule for different constrained ψ dihedral angles along the valley.

The whole path obtained by taking point A as reference is presented in Fig. 4; it diverges from the lowest energy valley after passing through the local minimum point C, and reaches a maximum energy point X. This divergence is due to the fact that the mrmsd from A does not increase isotropically in the (ϕ,ψ) conformational space, reaching a high value at point X, as shown in Fig. 4.

In order to solve this problem, a third variant was considered for the PEDC method; it consisted in changing the reference for each displaced structure. A given structure is displaced by taking as reference the one before it on the path (e.g. the reference structure for structure J is $J-2$). The path obtained with this variant is closer to the lowest energy path between points A and B, in comparison with the paths obtained with a fixed reference structure (Fig. 3b), and the whole path passes through the energy minima,

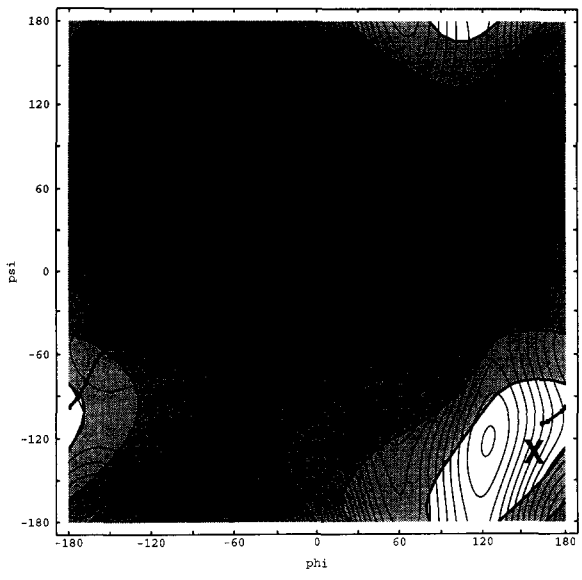

Fig. 4. Entire (ϕ,ψ) adiabatic energy map of N-methyl-alanyl-acetamide. Thin contours correspond to isoenergies as shown in Fig. 3; thick contours correspond to mrmsd isovalues from reference A and are shaded such that the lighter the shading the larger the mrmsd value. The path on this map corresponds to that determined by PEDC method which takes point A as reference.

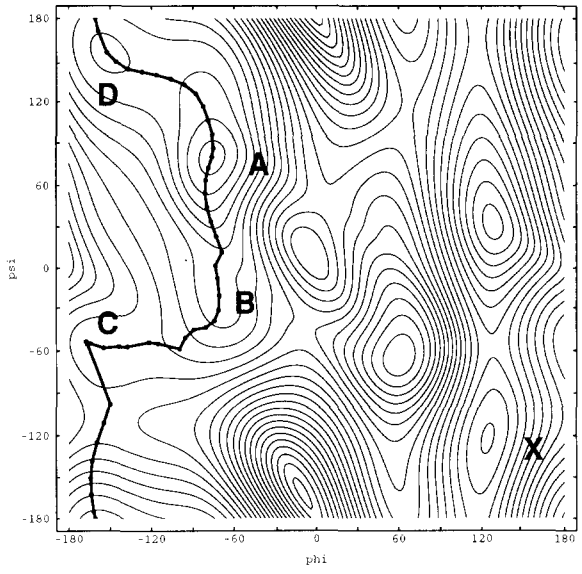

Fig. 5. (ϕ,ψ) adiabatic energy map of N-methyl-alanyl-acetamide containing the path determined with the PEDC method with a mobile reference algorithm (see text).

satisfactorily, as shown in Fig. 5.

3.2. Hemoglobin

We illustrate in this section the application of the PEDC method to the search for a path between the T and R states of fully oxygenated hemoglobin, by using the algorithm described in Fig. 1 (case B) with energy minimizations.

Human adult hemoglobin (Hb A) is a tetramer constituted of two α- and two β-chains. The α-chains are constituted of 141 residues, and the β-chains of 146 residues; each chain contains an iron heme. The protein is allosteric and it undergoes large conformational changes upon ligand binding (O_2, CO,...). Indeed, the crystallographically known structures of the unliganded T-state [13], and the fully liganded R-state [14] show a 15° rotation of the dimer $\alpha_2\beta_2$ with respect to the dimer $\alpha_1\beta_1$ inducing an mrmsd between T and R of 2.8 Å.

As the crystal structure of the fully oxygenated T-form of hemoglobin does not exist, the PEDC method with minimization was first used to obtain a model of the fully oxygenated T-state structure. The energy minimized structure of liganded R-state was progressively displaced toward the T-state structure which was used as a reference; the distance constraint was defined on a selection of atoms which excludes the ligands. The liganded T-state structure thus obtained remains very close, when minimized without any constraint, to the unliganded T-state crystal structure.

The PEDC method with minimization was then used, in a second stage, for determining the path between the fully oxygenated T- and R-states structures as obtained above. The path was obtained by departing from the T-structure, and using the R-structure as reference. Energy minimizations with the Adopted Basis Newton Raphson technique (ABNR) [12] were used along the path, with force constants $k_{\text{dist}} = 10^5$ kcal/mol/Å^2, $k_{\text{trans}} = 10^4$ kcal/mol/Å^2, and $k_{\text{rot}} = 10^{-6}$ kcal/mol/Å^4 a.u. The step size of the displacement was $\Delta d = 0.1$ Å, and the reference mrmsd d_0^J was decreased from 2.8 Å to 0.

The potential energy (V_{int}) obtained along the path between the T- and R-state structures is shown in Fig. 6. After a small increase down to an mrmsd of 1.5 Å from R-state, the potential energy decreases to a minimum at a distance of 1 Å. A structural analysis showed that this minimum corresponds to an R-

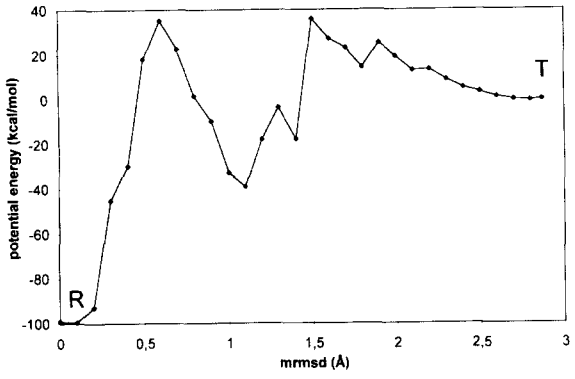

Fig. 6. Relative internal potential energy as a function of mrmsd from the R-structure along the transition path between the fully oxygenated T and R-state structures of hemoglobin. The zero energy corresponds to the starting T-state structure. The path was obtained with the PEDC method taking as reference the R-state and using energy minimizations.

quaternary and a T-ternary structure. This structure is separated from the R-state structure (last point on the path) by an important energy barrier which corresponds mainly to the energy required to reorient the side-chains to their proper R-state conformations. The observed barriers would be of lower magnitude if the simulations were carried out with molecular dynamics. The results obtained with molecular dynamics will be reported in a forthcoming article.

The calculations presented above were carried out on an SGI computer (R4400) and required 150 hours of CPU time.

3.3. *Phosphoglycerate kinase*

Yeast phosphoglycerate kinase (PGK) is a monomeric protein constituted of 415 residues folded in two globular domains; in the glycolytic pathway it catalyzes the first reaction that produces ATP with the formation of 3-phosphoglycerate (3PG). The crystallographic structures of unliganded and binary complexes of the PGK of different species are in the open form [15,16] (Fig. 7a), while the ternary complex structure is still unresolved. In the open form, the phosphate groups of the substrates are separated by a distance of $\approx$ 12 Å, while to have an in-line phosphoryl transfer between the two substrates, this distance should be less than 5 Å. In order to bring the substrates together, a hinge-bending motion should take place. With the PEDC method, we try to find a closed form of the structure, and see if it is compatible with the geometrical requirement for a phosphoryl transfer.

To constitute the ternary complex, the 3PG was positioned on the yeast binary complex structure PGK-MgATP, according to its position in the pig binary complex PGK-3PG [17]. For this application the parameter set 19 of CHARMM was considered. The electrostatic potential energy was evaluated with a variable dielectric constant equal to the interatomic distance for the pairs of atoms considered. A switching function with cut-on and cut-off distances equal to 10 Å and 12 Å, respectively, was considered for both the electrostatic and Van der Waals energies.

The energy of PGK-MgATP-3PG was minimized using the ABNR algorithm, then it was displaced to an mrmsd of 0.6 Å with the PEDC minimization procedure. The constrained molecular dynamics simulations were then started from this structure, using a distance constraint potential corresponding to Eq. (6); the force constants were $k_{\text{dist}} = 10^3$ kcal/mol/Å^2, $k_{\text{trans}} = 10^4$ kcal/mol/Å^2, and $k_{\text{rot}} = 10^{-4}$ kcal/mol/Å^4 a.u. A heating period of 10 ps was considered to reach the temperature of 300 K which was followed by molecular dynamics simulations at this temperature with periodic rescaling of velocities until the mrmsd reaches a value of $d_0^M = 8$ Å. The d_0^J value was increased by 0.1 Å every 0.5 ps during the simulations. The calculations were performed on a C98 Cray computer, and required 30 hours of CPU time in order to determine the whole path.

The potential energy, V_{int}, and the distance constraint energy, V_{dist}, vs the mrmsd are shown in Fig. 8a and b, respectively. A displacement of 1 Å occurred during the heating period in which the potential energy increased. Up to a distance of 2.3 Å and between 4.3 and 5.5 Å the constraint forces are weak; they become more important for larger distances. The internal potential energy decreases with small fluctuations and reaches a plateau with the gradual increase of the mrmsd. This variation is concomitant with a decrease of the radius of gyration of the molecule as shown in Fig. 9a; it corresponds to a hinge-bending closure of the domains. Experimentally a value of 1 Å reduction was observed in the radius of gyration of PGK upon formation of the ternary complex, by small angle x-ray scattering measurements [18] The closed form of PGK corresponding to an mrmsd of 7 Å is presented

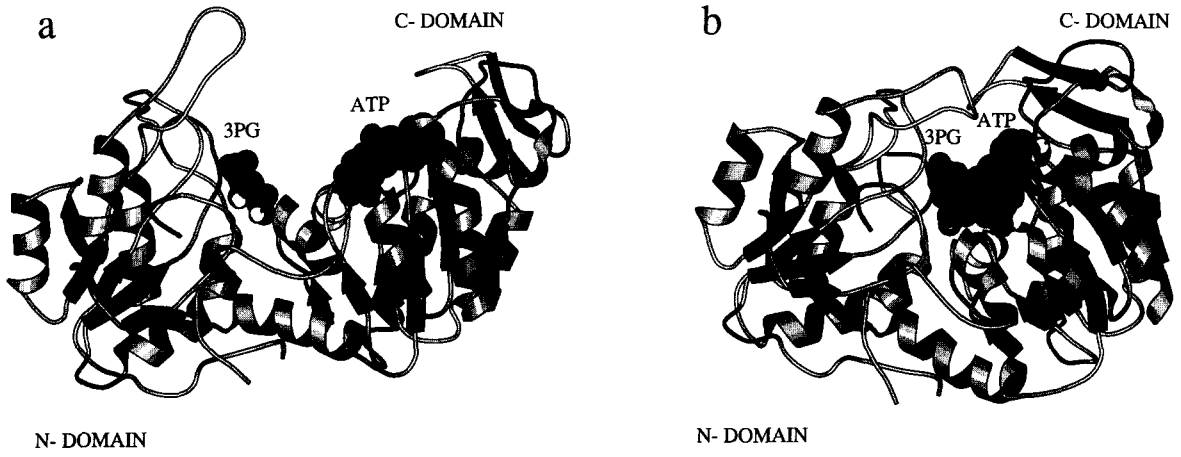

Fig. 7. Structures of yeast PGK: (a) open form corresponding to the energy minimized X-ray structure; (b) closed form obtained by the PEDC method. The substrates are represented in CPK model.

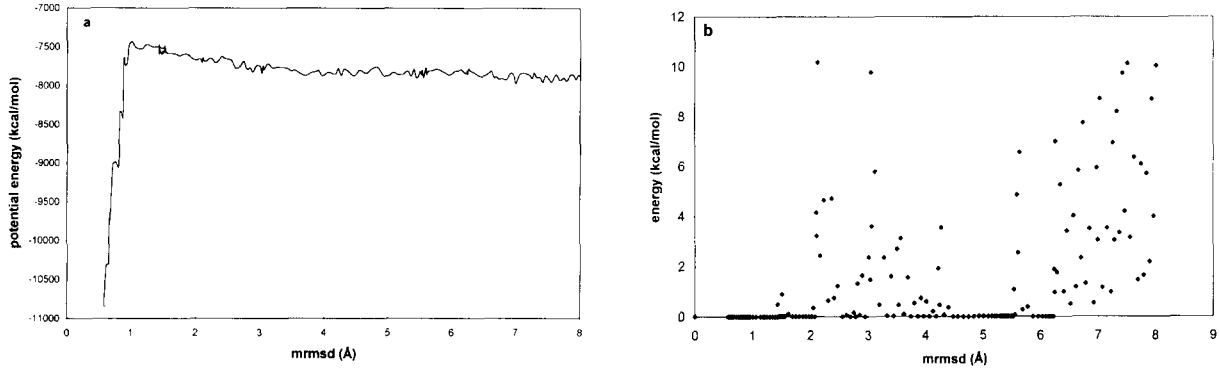

Fig. 8. PGK. (a) Internal potential energy as a function of mrmsd from the open structure during the molecular dynamics; (b) corresponding variations of the distance constraint energy.

in Fig. 7b.

The observed distances between the P_γ atom of ATP and the carboxylate group of 3PG, to which the phosphate group will be transferred, are shown in Fig. 9b. They show that when the protein reaches an mrmsd of 6 Å and beyond, the two substrates are close enough to allow the catalytic reaction, and are protected from the aqueous medium. An interesting point to notice in our results is that a 2 Å decrease of the radius of gyration from the open structure seems sufficient to lead to a catalytically favorable configuration of the substrates.

The definition of V^J_{dist} given in Eq. (6) was preferred to that given in Eq. (2) for carrying out distance constrained molecular dynamics simulations at 300 K. Indeed the latter definition led us to unrealistic structures presenting unusual distortion of the backbone due mainly to electrostatic interactions between charged groups, which were too strong in our model. When the charges of ionized groups are screened artificially by a factor 0.3, these distortions disappear. The use of the constraint potential V^J_{dist} in Eq. (6) does not introduce, even with full charges, such deviations of the structure.

Full results on PGK will be presented in a separate article.

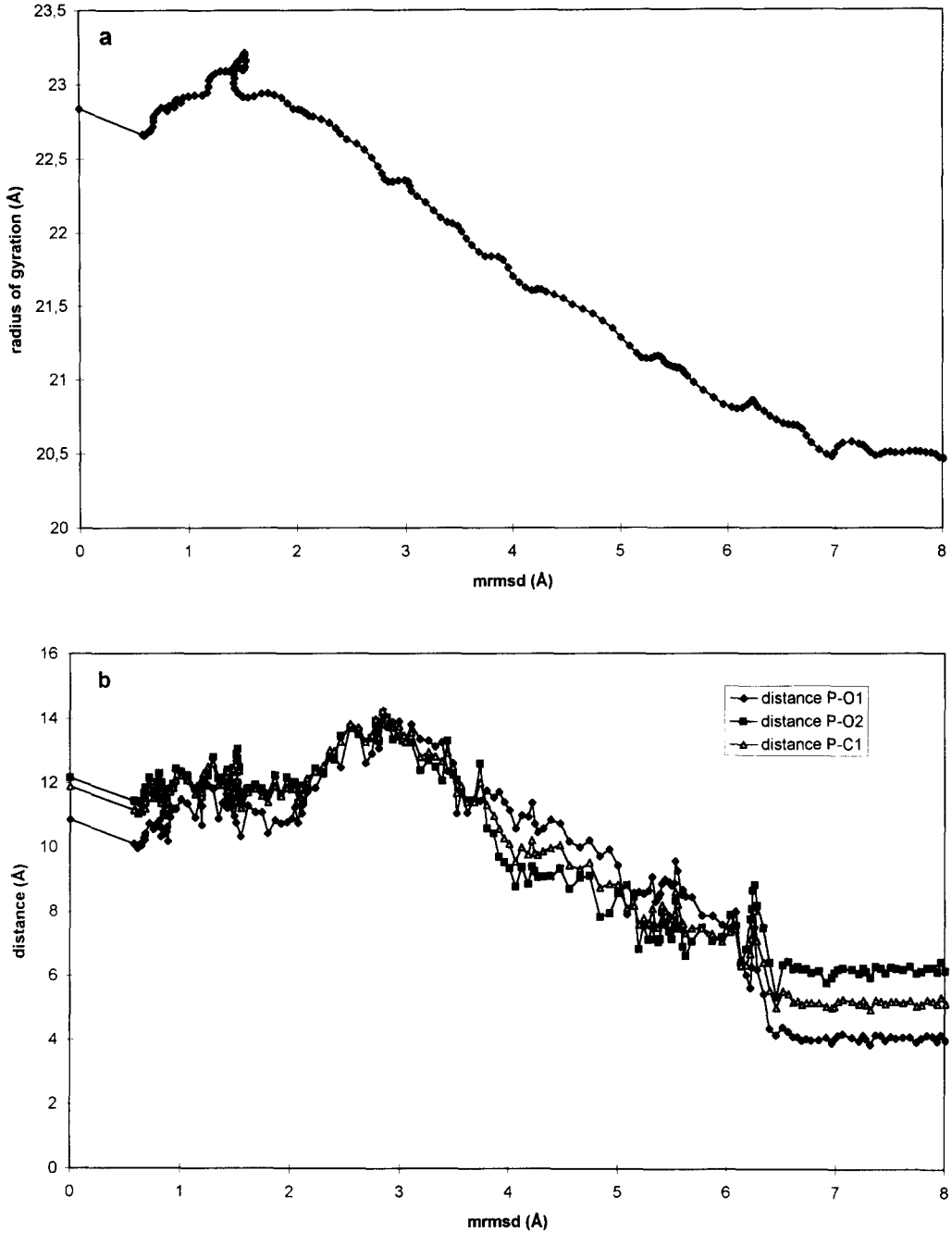

Fig. 9. PGK. (a) Radius of gyration as a function of mrmsd during the molecular dynamics; (b) corresponding variations of the distances between both ligands.

3.4. Discussion on the initial direction of displacement

In the examples presented above, the lowest frequency mode corresponded in each case to a functional variation of the molecule; in the case of PGK it corresponded to the closure of domains, and in hemoglobin to the T–R transition. However it may happen that such a functional transition may correspond to higher frequency modes, or one may be interested in exploring other directions. Test calculations performed with the above macromolecules showed that (results not reported) the paths initiated along directions corresponding to higher frequency modes, had a tendency to switch to the pathway obtained by following the lowest frequency mode. Thus our method does not allow conformational explorations in higher energy valleys. One possibility, however, for such explorations, is to displace the structure with a large step size along the chosen normal mode, and start the PEDC algorithm from this point.

3.5. Discussion on the force constant values of constraint potentials

A good choice of values for the force constant parameters for the constraint potentials depends on the size of the molecules considered. Several trial computations should be performed in order to have appropriate values for them which do not push the system out too much from the low energy valleys. Some general comments can be formulated about the force constant parameters based on our test calculations: (1) K_{rot} and K_{trans} are essential for forbidding global movements in small systems, but are less important in large systems; (2) in small systems, an increase of the K_{trans} value does not influence the path, whereas an increase of K_{rot} and K_{dist} values may have an important effect; (3) in macromolecules, an increase of K_{rot} and K_{trans} values does not influence the pathway significantly, but K_{dist} value in PEDC minimizations may affect the pathway if the system has to move backward (lowering the mrmsd from the starting structure) in order to adopt an energetically more favorable configuration; with molecular dynamics this effect is reduced because of fluctuations in the structure.

4. Conclusion

The results given above, although preliminary, show that the PEDC method may be applied to a wide variety of conformational analysis problems, and is well adapted for treating large molecular systems. The method used with energy minimizations, is able, in a systematic manner, to find from a single conformation other low energy points on the potential energy surface with no a priori knowledge where they might be located. However, as energy minimizations do not allow a large structural flexibility, it may be more appropriate to use the PEDC method with molecular dynamics in order to obtain paths presenting lower energy barriers.

The PEDC method may be used in conjunction with other methods, like the Conjugate Peak Gradient method [9], in order to better refine the transition paths obtained.

Acknowledgements

We are grateful to Thomas Simonson and Martin Field for useful discussions and considerable help with the manuscript. Some of the simulations were performed on the Cray C98 of the IDRIS supercomputer center of the C.N.R.S. (France). This work was supported by a 'Contrat de Recherche Externe' of INSERM (France) and by the Centre National de la Recherche Scientifique.

References

[1] W.S. Bennett and T.A. Steitz, Proc. Natl. Acad. Sci. USA 75 (1978) 4848–4852.
[2] W.S. Bennett and T.A. Steitz, J. Mol. Biol. 140 (1980) 211–230.
[3] M.F. Perutz, Nature. 228 (1970) 726–739.
[4] C.J. Cerjan and W.H. Miller, J. Chem. Phys. 75 (1981) 2800–2806.
[5] D.T. Nguyen and D.A. Case, J. Phys. Chem. 89 (1985) 4020–4026.
[6] R. Elber and M. Karplus, Chem. Phys. Lett. 139 (1987) 375–380.
[7] A. Ulitsky and R. Elber, J. Chem. Phys. 92 (1990) 1510–1511.
[8] M.A.E.-C. El-Kettani and J. Durup, Biopolymers 32 (1992) 561–574.

[9] S. Fischer and M. Karplus, Chem. Phys. Lett. 194 (1992) 252–261.
[10] S.C. Harvey and H.A. Gabb, Biopolymers 33 (1993) 1167–1172.
[11] D. Perahia and L. Mouawad, Comp. and Chem., in press.
[12] B. Brooks, R. Bruccoleri, B. Olafson, D. States, S. Swaminathan and M. Karplus, J. Comp. Chem. 4 (1983) 187–217.
[13] G. Fermi, M.F. Perutz, B. Shaanan and R. Fourme, J. Mol. Biol. 175 (1984) 159–174.
[14] B. Shaanan, J. Mol. Biol. 171 (1983) 31–59.
[15] R.D. Banks, C.C.F. Blake, P.R. Evans, R. Huber, D.W. Rice, G.W. Hardy, M. Merrett and A.W. Phillips, Nature. 279 (1979) 773–777.
[16] H.C. Watson, N.P.C. Walker, P.J. Shaw, T.N. Bryant, P.I. Wendell, I.A. Fothergill, R.E. Perkins, S.C. Conroy, M.J. Dobson, M.F. Tuite, A.J. Kingsman and S.M. Kingsman, EMBO J. 1 (1982) 1635–1640.
[17] K. Harlos, M. Vas and C.F. Blake, Proteins 12 (1992) 133–144.
[18] C.A. Pickover, D.B. McKay, D.M. Engelman and T.A. Steitz, J. Biol. Chem. 254 (1979) 11323–11329.