Molecular dynamics study of conformational changes in human serum albumin by binding of fatty acids

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Human serum albumin (HSA) is a major protein component of blood plasma. Binding of drugs to HSA is one of the most important factors determining pharmacokinetics of drugs (Kragh-Hansen *et al.*, 2002). When measuring binding affinity of a drug to HSA *in vitro*, defatted HSA is usually used. On the other hand, under normal physiological conditions, HSA binds with fatty acid. So far, there is little information on conformational changes of HSA upon binding of fatty acids. The present study was aimed to elucidate the conformational changes as well as the structure and dynamics of HSA, based on the molecular dynamics (MD) simulations with explicit water molecules.

Materials and methods. The initial coordinates of unliganded HSA and HSA-myristate (HSA-MYR) complex were obtained from Protein Data Bank (unliganded HSA: PDB entry 1AO6, HSA-MYR: PDB entry 1BJ5). A series of MD calculations were carried out using AMBER7 package (Case *et al.* 2002). A rectangular-shaped box of water was constructed. 5 ns MD calculations were carried out for both the unliganded HSA and HSA-MYR complex models under periodic boundary condition. The long-range electrostatic interactions were handled by the particle mesh Ewald algorithm (Darden *et al.*, 1993). The resultant model systems contained 87223 (unliganded HSA) and 99126 (HSA-MYR complex) atoms, respectively.

Results and discussion. The root mean square deviation (RMSD) from the X-ray structure over the course of a MD simulation reached plateau at about 2 ns. The RMSD values were as small as about 3.0 Å, which were roughly comparable to the X-ray resolution. Hence, we concluded that significant structural drift from the X-ray structure did not occur during the MD simulations.

Binding of MYR to HSA increased the radius of gyration (Rg) of HSA in the MD simulations. Through the structural comparison of the average structures, the dramatic extent of the relative motions of domains I and III, especially those of subdomains IA and IIIB, were observed. Thus, increase in Rg by binding of MYR molecules should be observed for HSA, as a result of the motions of domains I and III.

Local protein mobility was analyzed by calculating the time-averaged root mean square fluctuation (RMSF) for each residue, using the trajectory in an equilibrium state. RMSF values at drug binding sites I (subdomain IIA) and II (subdomain IIIA) were increased by binding of MYR. This result implies that binding affinity of drugs at these primary binding sites can be changed by MYR binding.

To analyze internal motions of the whole HSA molecule and each domain, principal component analysis for collective coordinates from MD simulations was carried out. The primary internal motions, characterized by the first and the second principal components, PC1 and PC2, were observed mainly at domains I and III. The directional motion projected on PC1 of unliganded HSA was similar with that projected on PC2 of HSA-MYR complex, indicating that the first principal directional motion in unliganded HSA is conserved as the second principal directional motion in HSA-MYR complex. On the other hand, the second principal directional motion in unliganded HSA partially turned into the first principal directional motion in HSA-MYR complex.

Conclusion. The present study unraveled possible conformational changes in aqueous solution caused by binding of MYR molecules to HSA, based on the results of the MD simulations.

Case, D.A., Pearlman, D.A., Caldwell, J.W., Cheatham, T.E. 3rd, Wang, J., Ross, W.S., Simmerling, C.L., Darden, T.A., Merz, K.M., Stanton, R.V., Cheng, A.L., Vincent, J.J., Crowley, M., Tsui, V., Gohlke, H., Radmer, R.J., Duan, Y., Petera, J., Massova, I., Seibel, G.L., Singh, U.C., Weiner, P.K., Kollman, P.A. (2002) AMBER7. San Francisco: University of California.

Darden, T., York, D., Pedersen, L. (1993) *Journal of Chemical Physics* 98, 10089-10092.

Kragh-Hansen, U., Chuang, V.T.G., Otagiri, M. (2002) *Biological and Pharmaceutical Bulletin* 25, 695-704.