A. Introduction

The simulation of the motion of macromolecules in realistic environments is made possible by the technique of molecular dynamics. Prior to application of the method is the development of a potential energy surface, or “force field”, from which the forces on all atoms of the system can be derived. This force field is typically parameterized by resorting to spectroscopic methods and ab initio quantum mechanical computations on smaller molecules. Once the force field has been developed, Newton’s equations of motion can be integrated to give the trajectory of the molecule. Most calculations on proteins to date have involved integrations of 20–200 ps, although we note a recent simulation of 550 ps on hen egg white lysozyme (Mark and van Gunsteren 1992). It is probably reasonable to state that the current level of simulation technique allows one to examine the early stages of unfolding events that might be anticipated as a molecule is solvated from a crystalline environment. Many aspects of the methodology are adequately discussed in recent reviews by van Gunsteren and Mark (1992) and Karplus and Petsko (1990).

In this contribution we review our application of the molecular dynamics method to the H-ras p21 system based on the 1.35-Å resolution crystal coordinates of Pai et al. (1990). This paper is an extension of our earlier work (Foley et al. 1992) on this system. The p21–GTP system represents a substantial challenge for simulation in that the molecule and its ligands (GTP, Mg^{2+} ion) are highly charged and there are no disulfide bonds present to provide intrinsic stabilization. Other theoretical studies have been carried out by Dykes et al. (1992) based on the alpha carbon coordinates of the Tong et al. (1991) structure of p21–GTP and by Prive et al. (1992), who attempt to model the transition state of the hydrolysis of p21–GTP based on their crystal structure of p21–GTP (analog) and a similar crystal structure of the mutant protein in the GTP-bound state. The essential biological details of this molecule are more than adequately discussed in other papers of this volume; however, we do emphasize that our simulations pertain to the structure of p21–GTP which is responsible for the intrinsic GTP hydrolysis process.
B. Methods

All energy minimization and molecular dynamics computations were carried out with the AMBER 3A program (Weiner et al., 1984, 1986; Seibel 1989) using the 1.35-Å resolution x-ray crystallographic coordinates of H-ras p21 (residues 1-166) of PAI et al. (1990). The all-atom force field (Weiner et al. 1986) was employed for all calculations. A 9-Å nonbonded cutoff was employed for the energy minimization; however, a twin-range cutoff (9 Å, 22 Å) proved necessary for all dynamics calculations. A rectangular box of approximately 9500 water molecules of the TIP3P description (Jorgensen et al. 1983) was used to solvate the protein. A mass of 1 amu (atomic mass unit) was used for all hydrogen atoms and all bonds involving hydrogen were constrained (see below). A step length of 1 fs was used throughout with the nonbonded list updated every ten integration steps. At each list update, the force was corrected to include electrostatic and attractive van der Waals interactions in the range of 9.0–22.0 Å. A parallel computation utilizing only a single 9-Å cutoff, but with all other procedures the same, was performed to fully assess the importance of the twin-range cutoff. The frequent nonbonded list update and the twin cutoff were found to be necessary in this system to equilibrate the RMS deviation (simulation structure vs. crystal structure). Similar conclusions resulted from our simulations (TAD, PSC, LGP) performed on the calcium-bound bovine prothrombin fragment-1 molecular system, a highly charged ionic system (Hamaguchi et al. 1992). In the case of prothrombin, the twin cutoff was essential for the retention of an important ionic interaction in which the N terminus was folded back onto the protein. The simulations were performed at 300K after very careful initial ramping of the temperature over the first 6 ps of the simulation. Care was taken to insure that the protein and water bath exhibited the same average temperatures throughout the simulations.

The crystal structure (PAI et al. 1990) was determined with a very slowly hydrolyzing GTP analog in which an isoelectronic N-H group replaced the ethereal oxygen between the γ- and β-phosphate functional groups. The simulations were performed with the physiologically relevant oxygen in place. A single proton was added to the oxygen on the GTP γ-phosphate group that was not involved in any hydrogen-bonding interaction with the protein. The point charge parameters for the GTP were obtained by fitting the ab initio quantum mechanical electrostatic potential at the 3-21G* basis level (charges scaled to the 6-31G** level). The ab initio quantum chemical calculation was performed with the Gaussian 90 program (Frisch et al. 1990) and the subsequent electrostatic potential calculation was performed with the CHELP code (Chirlian and Francl 1987a,b). Computations were carried out on Cray Y-MPs at the National Cancer Center (Frederick, MD) and the North Carolina Supercomputing Center (Research Triangle Park, NC). Graphics analysis capability was provided by the MULTI program (Darden et al. 1991) on Silicon Graphics workstations.