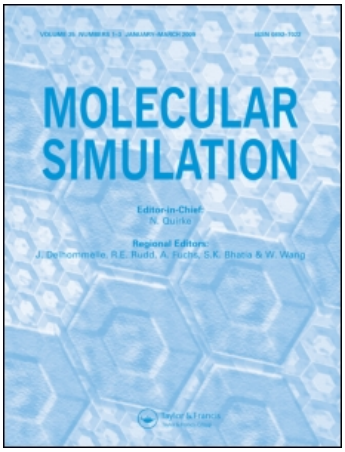

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On the Approximation of Solvent Effects on the Conformation and Dynamics of Cyclosporin A by Stochastic Dynamics Simulation Techniques

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ON THE APPROXIMATION OF SOLVENT EFFECTS ON THE CONFORMATION AND DYNAMICS OF CYCLOSPORIN A BY STOCHASTIC DYNAMICS SIMULATION TECHNIQUES

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The molecular simulation technique of stochastic dynamics (SD) is tested by application to the immunosuppressive drug cyclosporin A (CPA). Two stochastic dynamics simulations are performed, one (SD_{CCl_4}) with atomic friction coefficients proportional to the viscosity of the nonpolar solvent CCl_4, and one (SD_{H_2O}) with atomic friction coefficients corresponding to an aqueous solution. The atomic friction coefficients are also taken proportional to an approximate expression for the atomic accessible surface area. The properties of both stochastic dynamics simulations are compared to those of two full molecular dynamics (MD) simulations of cyclosporin A, one in a box with 591 CCl_4 molecules, and one in a box with 632 H_2O molecules.

The properties of cyclosporin A as found in the molecular dynamics simulation in CCl_4 are well reproduced by the SD_{CCl_4} simulation. This indicates that the neglect of a mean force reresenting the average solvent effects on the solute is justified in the case of nonpolar solvents. For polar solvents, like water, this mean force may not be neglected. The SD_{H_2O} simulation of cyclosporin A clearly fails to reproduce the amount of hydrogen bonding found in the molecular dynamics stimulation of cyclosporin A in water.

A comparison with a molecular dynamics simulation of cyclosporin A *in vacuo* shows that both the SD_{CCl_4} and the SD_{H_2O} simulation come closer to the properties of the molecular dynamics simulations in CCl_4 and in H_2O than a molecular dynamics simulation *in vacuo*.

KEY WORDS: Stochastic dynamics, solvent effects, cyclosporin A, molecular simulation, potential of mean force

INTRODUCTION

Molecular simulation of many-particle systems, such as liquids, solutions and macromolecules, has become a widely used technique that is applied to a variety of physical and chemical systems. In the molecular dynamics (MD) method [1] Newton's classical equations of motion

$$dx_i(t)/dt = v_i(t) \qquad (1.1)$$

$$m_i \, dv_i(t)/dt = F_i(\{x_i(t)\}) \tag{1.2}$$

are solved for a system of N particles. The index i labels particles and cartesian components ($i = 1,2,\ldots,3N$). The x-, y-, or z-coordinate of a particle is denoted by x_i, its mass by m_i and the cartesian components of the velocity by v_i. The (systematic) force F_i is to be derived from a conservative force field $V(\{x_i\})$

$$F_i(t) = -\partial V(\{X_i\})/\partial X_i. \tag{1.3}$$

This potential function V describes the interaction between the particles, which generally depends on the coordinates of all particles, denoted by $\{x_i(t)\}$.

When modelling macromolecular systems using molecular dynamics, practical limitations of computational effort form a strong incentive to reduce the number of degrees of freedom as much as possible, while retaining a truthful simulation of the physical characteristics of interest. In principal there are two ways to achieve this reduction.

a. Hard degrees of freedom, that is, those corresponding to high-frequency normal modes, can be treated as if they were completely constrained and thus eliminated from the system. This approach leads to constrained dynamics, which increases the computational efficiency by allowing for a larger time step in the simulation [2].
b. Less relevant degrees of freedom can be ignored and their influence on the remaining part of the system approximated by a combination of mean force interactions, stochastic and frictional forces. This approach leads to stochastic dynamics (SD).

When simulating a (macro) molecule in solution, one is generally not interested in the details of the motion of the solvent moleules. Yet, in an aqueous solution of the number of solvent atoms is generally at least 10 times larger than the number of solute atoms [3,4]. This is due to the fact that each water molecule contains three atoms, and many water molecules are required to fill the remaining space of the (periodic) computational box containing the solute molecule. Therefore one would like to get rid of explicit treatment of solvent molecules in the simulation, but would like to maintain their influence on the conformation and dynamics of the solute. This influence can be divided into three types.

a. The average or mean interaction between solute atoms is affected by the presence of solvent.
b. The solute atoms experience a frictional force due to the solvent.
c. The solute atoms experience a randomly fluctuating force due to collisions with solvent atoms.

The average effect of solvent atoms labelled by the index α on the interaction between solute atoms denoted by the index i can be incorporated in the interaction function $V_{mean}(\{x_i\})$ for the solute by averaging over the solvent (α) degrees of freedom in an equilibrium ensemble.

$$v_{mean}(\{x_i\}) = \langle V(\{x_i\},\{x_\alpha\}) \rangle_\alpha. \tag{1.4}$$

The index i now runs over the N_ρ solute degrees of freedom. The function $V_{mean}(\{x_i\})$ is called a potential of mean force [5]. For liquid n-butane the average effect of the solvent molecules on the solute conformation can be clearly observed [6,7]. The distribution of the torsional angle shows in the liquid phase an enhancement of the population of the gauche conformations over that of the trans conformation, when compared to the gas phase. This mean effect of the solvent can be

understood from packing and steric effects. However, for longer n-alkanes, like n-decane, the mean effect of the solvent on the solute conformation and dynamics was found to be very small [8]. One would indeed expect that for nonpolar organic solvents the mean force due to the solvent plays a minor role in determining solute conformation. But for polar solvents, like water, a considerable contribution to the mean interaction is expected, especially when the solute contains charged or polar groups of atoms. A few phenomenological potentials of mean force for polypeptides in aqueous solution have been proposed [9–11]. The accessible area model of Eisenberg and McLachlan [11] takes $V_{\text{mean}}(\{x_i\})$ proportional to the accessible surface area of the individual atoms of the solute, using a weight function accounting for the type (charged, polar, nonpolar, etc.) of atom. The shell model of Scheraga and coworkers [9,10] goes one step further by also accounting for the possibility of hydrogen bond formation between solute and solvent.

The two other types of solvent influence can be mimiced by the inclusion of a frictional force and a random force in Newton's equations of motion. In the simplest case the frictional force is proportional to the velocity of the atom to which it applies, and the random force is of white noise character, uncorrelated between the various degrees of freedom:

$$m_i \, \mathrm{d}v_i(t)/\mathrm{d}t \;=\; F_i(\{x_i\}) \;-\; m_i \, \gamma_i \, v_i(t) \;+\; R_i(t). \tag{1.5}$$

This equation is called the Langevin equation. The atomic friction coefficient is denoted by γ_i. The systematic force F_i is to be derived from the interaction function $V_{\text{mean}}(\{x_i\})$ and the random force is denoted by R_i. It is assumed to be a zero-mean gaussian-distributed random variable and to have no correlation with prior velocities, nor with the systematic force. The width of the gaussian distribution of R_i is related to the friction coefficient γ_i by the second fluctuation-dissipation theorem [12]:

$$< R_i(0) \, R_j(t) > \;=\; 2 \, m_i \gamma_i \, k \, T_{\text{ref}} \, \delta_{ij} \, \delta(t). \tag{1.6}$$

Boltzmann's constant is denoted by k and T_{ref} is the reference temperature of the solvent bath. Various algorithms for efficient integration of the Langevin equation (1.5) have been proposed [13–15].

The choice of appropriate atomic friction coefficients γ_i will in general depend upon the type of system that is considered. For small solutes, like butane and decane, the friction coefficient may be taken equal for all atoms in the solute. Its size may be determined from the molecular diffusion constant D_{mol} using the relations.

$$D_{\text{mol}} \;=\; k \, T_{\text{ref}}/(M\gamma_{\text{mol}}) \tag{1.7}$$

and

$$\gamma_i \;=\; \gamma_{\text{mol}} \tag{1.8}$$

where M denotes the molecular mass and γ_{mol} the molecular friction coefficient [8]. Values for D_{mol} may be obtained from experiment or by molecular dynamics computer simulation of the appropriate liquid [8]. An alternative is to assume the validity of Stokes' law

$$\gamma \;=\; 6 \, \pi \, R \, \eta/m \tag{1.9}$$

where R denotes the Stokes' radius for an atom and η is the viscosity of the solvent [16]. In larger molecule, like proteins, not all atoms are in contact with the solvent. In that case the atomic friction coefficient must depend on the degree of solute atom – solvent interaction. The atomic friction coefficient γ_i may be taken proportional to the accessible surface area of atom i.

When describing solute conformation and dynamics by stochastic dynamics techniques, the central question is how well the properties obtained by a full treatment of solute plus solvent by molecular dynamics will be reproduced in the simplified stochastic treatment. In order to shed some light on ths question we have performed two stochastic dynamics simulations of a cyclic undecapeptide, the immunosuppressive drug cyclosporin A (CPA) [17]. For this molecule full molecular dynamics simulations in water (polar environment) and in CCl_4 (nonpolar environment) are available [18,19]. The friction coefficients of the two stochastic dynamics simulations were chosen to correspond with H_2O and CCl_4 as solvents. A comparison of stochastic dynamics with molecular dynamics results will show the effects of the approximate treatment of the solvent in the former method. We will also compare the stochastic dynamics to those of a molecular dynamics simulation of cyclosporin A in vacuo [20]. Section 2 contains a description of the model and computational details. The results of the five simulations are compared in section 3 and section 4 contains a brief discussion of the conclusions that may be drawn from this study.

2. MODEL AND COMPUTATIONAL PROCEDURE

Cyclosporin A (CPA) is a cyclic undecapeptide (Figure 1), the structure of which can be described as follows [21]. Residues 1 to 7 form an antiparallel β-pleated sheet with a type II-β turn at residues 3 and 4. The remaining residues 7 to 11 form a loop containing a γ turn of type C_7. Residue 8 is a D-amino acid and residues 9 and 10 are connected by a cis amide bond. The conformation of cyclosporin A in solution is of interest because of its immunosuppressive properties [17].

Cyclosporin A was simulated using the GROMOS (GROningen MOlecular Simulation) program library [22]. The molecular model and force field are described in [22]. The potential energy describing the interaction between the N atoms is of the usual type applied to proteins. It is composed of terms representing bond angle bending, harmonic (out-of-plane, out-of-tetrahedral configuration) dihedral angle bending, sinusoidal dihedral torsion, van der Waals' and electrostatic (Coulomb) interactions. Bond lengths are kept rigid by applying the SHAKE method [23,24]. Hydrogen atoms attached to carbon atoms are incorporated into the latter to form united atoms, whereas all polar hydrogens are explicitly treated. The force field does not contain an explicit hydrogen bonding term. Hydrogen bonding is the result of the attractive and repulsive Coulomb forces and the repulsive van der Waals forces between the atoms of polar groups [25].

Some parameters of the five molecular dynamics simulations that are considered here, are listed in Table 1. The simulation of cyclosporin A in vacuo has been described in [18,20]. The two solvent simulations, one in CCl_4 and one in H_2O have been described in [18,19]. The nonpolar CCl_4 molecule is treated as a united atom using the interaction function parameters of Rebertus and Berne [26]. The water molecules are modeled by the simple three-point charge (SPC) model of Berendson *et al* [27]. Both solvent simulations were performed using truncated octahedron

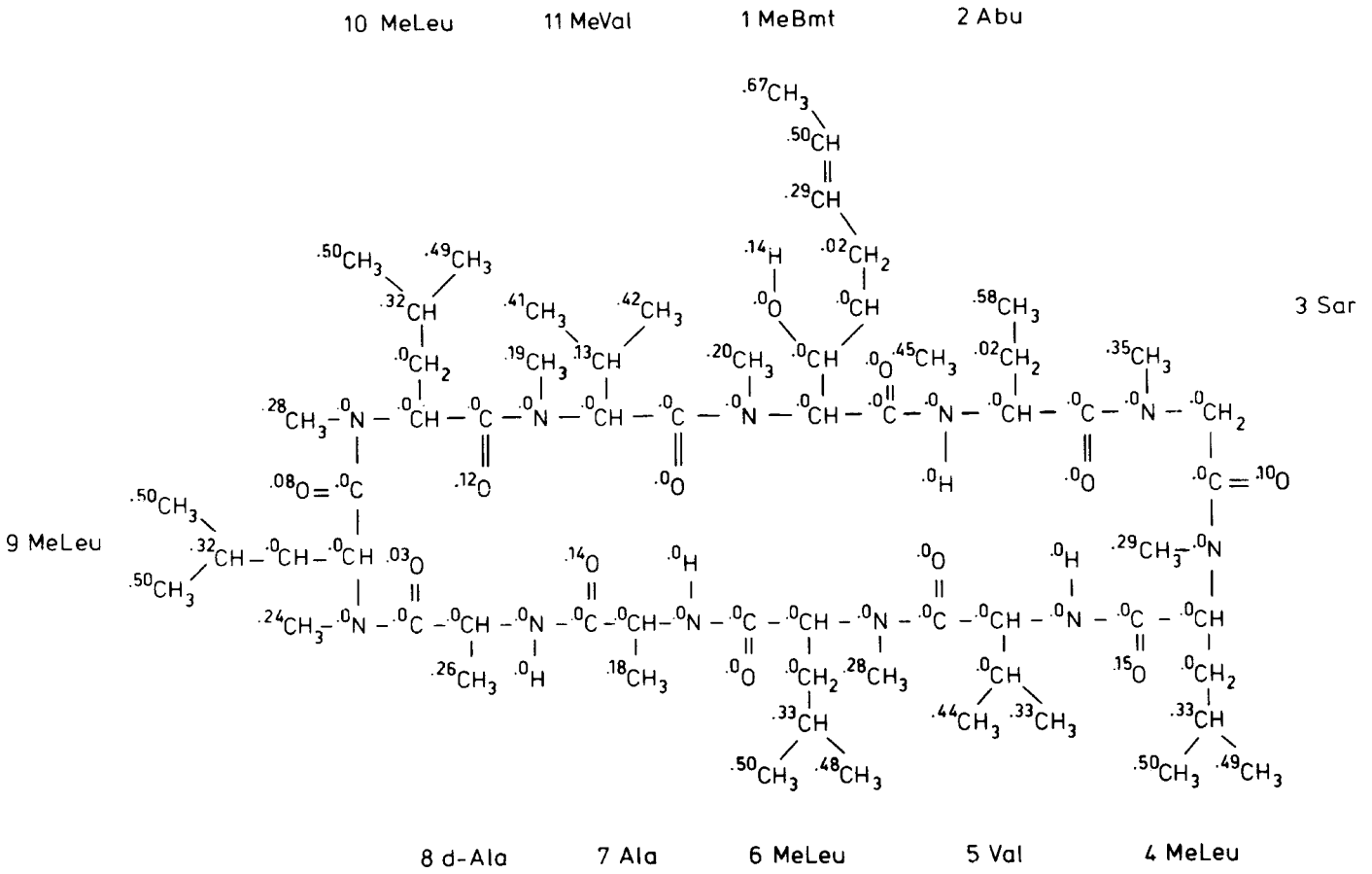

Figure 1 Primary structure of cyclosporin A. The time-averaged atomic accessible area weight factors $< \omega_i >$ for the SD_{CCl_4} simulation are listed with the atoms.

Table I Parameters and time-averaged energies of the CPA simulations

	MD in vacuo	*MD in CCl_4*	*SD_{CCl_4}*	*SD_{H_2O}*	*MD in H_2O*
N_{solute} [a]	90	90	90	90	90
$N_{solvent}$ [b]	0	591	0	0	632
γ_s [c] (ps^{-1})	–	–	24	91	–
$< E_b >$ [d] (kJ/mol)	–[f]	365	370	348	339
$< E_{nb} >$ [e] (kJ/mol)	–[f]	−150	−136	−139	−88
Length of run (ps)	40	50	50	50	45
CPU-time[g] (hr)	3.5	9.4	4.5	4.5	140

[a] N_{solute} = number of solute atoms.
[b] $N_{solvent}$ = number of solvent atoms.
[c] γ_s = solvent friction coefficient.
[d] $< E_b >$ = time-averaged bonded interaction energy.
[e] $< E_{nb} >$ = time-averaged nonbonded interaction energy.
[f] These energies were not given in reference [19].
[g] CPU-time on a VAX 11/780 per 10 ps of simulation.

periodic boundary conditions. Coupling to a temperature bath and a pressure bath using the method of [28] was applied to keep the systems at 300 *K* and 1 atm.

The parameters of the two stochastic dynamics simulations described here and denoted by the symbols SD_{CCl_4} and SD_{H_2O}, were chosen such that the stochastic dynamics simulations closely correspond to the molecular dynamics simulations in CCl_4 and in H_2O. As starting point for the simulations the same conformation of cyclosporin A was used as in [18,19]. The molecular model and interaction function were identical, like the time step of 2 femtoseconds and the relative geometric accuracy of SHAKE of 10^{-4}. In the molecular dynamics simulation in vacuo and the two stochastic dynamics simulations no cut-off radius R_{cut} was applied to the nonbonded interactions. Because of the periodic boundary conditions, the solvent MD simulations were performed using R_{cut} = 1.3 nm (CCl_4) and R_{cut} = 0.8 nm (H_2O).

The atomic friction coefficients γ_i for the 90 atoms of cyclosporin A were calculated using the formula

$$\gamma_i = \gamma_s \omega_i \tag{2.1}$$

where γ_s denotes the friction coefficient of the solvent molecules, which is derived from the experimental solvent viscosity η using Stokes' law (1.9), and ω_i is an atomic accessible area weight factor. For CCl_4 at 300 K the measured viscosity is η_{CCl_4} = 0.00881 P and for water at 300 K, η_{H_2O} = 0.008513 P [29]. The mass of a CCl_4 molecule is m_{CCl_4} = 153.823 a.m.u. and of a water molecule it is m_{H_2O} = 18.0154 a.m.u. The Stokes' radius R for a CCl_4 united atom was derived from the van der Waals' parameters C_{12} and C_6 ($C_{12}/r^{12} - C_6/r^6$) that were used in the MD simulation of cyclosporin A in CCl_4 [19], using the formula [30]:

$$R = 1/2\ (2\ C_{12}/C_6)^{1/6}. \tag{2.2}$$

One finds R_{CCl_4} = 0.369 nm and subsequently $\gamma_s = 24\,ps^{-1}$ for CCl_4. For water the molecular Stokes' radius R_{H_2O} = 0.170 nm was taken from [30], leading to a friction coefficient γ_s = 91 ps^{-1} for H_2O.

The atomic weight factors in (2.1) should be taken proportional to the accessible surface area of each solute atom. However, the calculation of this quantity, either numerically following Lee and Richards [31] or analytically following Richmond [32], is not a trivial task. Therefore, we approximate this quantity by taking

$$\omega_i = \text{maximum } (0, 1 - N_i^{nb}/N^{nbref}). \quad (2.3)$$

The number of neighbour atoms of atom i within a sphere of radius R^{nbref} is denoted by N_i^{nb} and N^{bref} should be chosen to be the number of neighbours at which atom i looses its contact with the solvent. In both stochastic dynamics simulations we chose $R^{nbref} = 0.3$ nm and $N^{nbref} = 6$. This choice leads to time-averaged weight factors, $< \omega_i >$, that are displayed in Figure 1 for the SD_{CCl_4} simulation. One can observe that atoms at the inside of the cyclosporin A molecule have $< \omega_i > = 0$, whereas the $< \omega_i >$ values increase when an atom is more exposed to solvent. During the stochastic dynamics simulations the ω_i values were recalculated from the actual cyclosporin A structure only after every picosecond, since they will be a slowly varying function of the molecular conformation of the cyclosporin A molecule. The time-averaged atomic weight factors $< \omega_i >$ are in the SD_{H_2O} simulation very comparable to those in the SD_{CCl_4} simulation; the root mean square difference is only 0.024. The mean over all cyclosporin A atoms of $< \omega_i >$ is 0.14, both in the SD_{CCl_4} and in the SD_{H_2O} simulation.

The stochastic dynamics simulations covered 50 ps of which the final 40 ps were used for analysis, as in [18,19] for the molecular dynamics simulations in solvent. Configurations of the trajectories were stored every 0.05 ps.

Since cyclosporin A is a rather hydrophobic molecule, one may expect that the potential of mean force due to the nonpolar CCl_4 solvent is small and can be safely omitted from the calculation. For cyclosporin A in aqueous solution the potential of mean force due to the polar H_2O solvent is expected to have a significant effect on the solute properties. In order to test these expectations, we did not include extra terms representing the mean solvent effect in the interaction function of reference [22]. If the stochastic dynamics and molecular dynamics simulations yield corresponding results, the mean force was correctly ignored. The contrary will be true when significant differences are found.

3. RESULTS

The five simulations of cyclosporin A will be compared and discussed in terms of average molecular structure, atomic positional fluctuations and hydrogen bond patterns. To compare the dynamics a few atomic positional fluctuation time correlation functions are shown.

The root mean square atom positional differences between the five time-averaged

Table II Root mean square atom positional differences between various time-averaged conformations of CPA[a)]

	MD in vacuo	*MD in CCl_4*	*SD_{CCl_4}*	*SD_{H_2O}*	*MD in H_2O*
MD in vacuo	–	.019	.024	.037	.039
MD in CCl_4	.047	–	.010	.037	.037
SD_{CCl_4}	.049	.030	–	.033	.036
SD_{H_2O}	.076	.077	.067	–	.030
MD in H_2O	.101	.116	.104	.086	–

[a)] Upper right-hand side triangles: mean over C_α atoms.
Lower left-hand side triangle: mean over all atoms.
Units: nm.

Table III Atomic positional fluctuations in CPA

Root mean square atom positional fluctuation[a]	*MD in vacuo*	*MD in CCl_4*	*SD_{CCL_4}*	*SD_{H_2O}*	*MD in H_2O*
all atoms	.069	.067	.079	.072	.063
C_α atoms	.045	.042	.045	.033	.040
MeLeu C_β	.072	.067	.064	.046	.079
MeLeu C_γ	.089	.098	.095	.076	.073
MeLeu C_δ	.128	.139	.132	.089	.102
Anisotropy[b]					
all atoms	.37	.39	.39	.42	.38
C_α atoms	.41	.40	.42	.49	.43

[a] Units: nm. Mean over quoted atoms.
[b] Ratio of the shortest and longest axis of the atom positional fluctuation ellipsoids. Mean over quoted atoms.

Table IV Backbone torsional angles and fluctuations[a] in CPA

Angle		*MD in vacuo*	*MD in CCl_4*	*SD_{CCL_4}*	*SD_{H_2O}*	*MD in H_2O*
1MeBmt	ϕ	−101(11)	−101(9)	−104(9)	−107(9)	−115(10)
	ψ	95(11)	88(16)	88(13)	88(14)	83(16)
	ω	178(7)	179(9)	178(8)	−175(7)	−179(7)
2Abu	ϕ	−84(15)	−82(17)	−84(13)	−91(14)	−81(17)
	ψ	96(9)	96(9)	97(9)	102(10)	107(12)
	ω	−159(9)	−157(9)	−159(10)	−168(10)	−170(10)
3Sar	ϕ	56(10)	56(10)	56(11)	57(10)	56(11)
	ψ	−116(10)	−120(13)	−120(14)	−114(10)	−117(10)
	ω	168(8)	167(8)	168(9)	166(7)	168(8)
4MeLeu	ϕ	−113(10)	−115(11)	−115(10)	−114(9)	−116(9)
	ψ	33(18)	52(26)	47(26)	32(20)	54(18)
	ω	177(7)	176(9)	176(8)	178(7)	179(7)
5Val	ϕ	−88(22)	−98(24)	−94(23)	−94(20)	−112(19)
	ψ	120(10)	120(10)	116(10)	113(9)	116(9)
	ω	180(9)	−177(9)	−175(9)	−176(7)	178(8)
6MeLeu	ϕ	−90(11)	−92(10)	−94(10)	−102(9)	−94(10)
	ψ	96(11)	92(12)	88(12)	90(10)	105(13)
	ω	−179(6)	180(7)	−178(7)	−175(6)	180(8)
7Ala	ϕ	−90(14)	−91(14)	−89(13)	−85(12)	−113(17)
	ψ	66(13)	69(12)	72(12)	73(13)	80(16)
	ω	178(7)	180(7)	179(7)	177(6)	178(6)
8Ala	ϕ	80(17)	70(14)	74(16)	75(14)	79(17)
	ψ	−128(11)	−125(10)	−127(11)	−126(9)	−133(10)
	ω	−177(7)	−176(8)	−175(8)	−177(7)	−171(7)
9MeLeu	ϕ	−132(10)	−129(10)	−132(11)	−129(9)	−135(8)
	ψ	113(13)	114(11)	114(12)	116(13)	108(10)
	ω	−12(15)	−13(11)	−14(7)	−16(14)	−14(12)
10MeLeu	ϕ	−121(11)	−123(8)	−121(11)	−120(10)	−115(9)
	ψ	101(11)	96(9)	90(10)	98(9)	105(12)
	ω	−164(11)	−156(11)	−156(10)	−158(8)	−160(7)
11MeVal	ϕ	−124(8)	−122(8)	−120(8)	−121(8)	−123(9)
	ψ	104(12)	99(11)	97(12)	93(10)	95(11)
	ω	−172(12)	−164(14)	−160(12)	−159(9)	−157(12)

[a] Root mean square fluctuation between parentheses.
Units: degrees.

structures of cyclosporin A are listed in Table II, both as a mean over the C_α atoms and as a mean over all atoms. Near the diagonal the differences are smallest, they increase when moving off the diagonal. This means that the order in which the five simulations are displayed, corresponds to their mutual spatial differences. The average structure of the first three simulations, molecular dynamics in vacuo, molecular dynamics in CCl_4, and SD_{CCl_4}, are much alike. The average structure of SD_{H_2O} differs more from those of the other simulations and the molecular dynamics simulation in water yields the most deviating average structure.

The atomic positional fluctuations are given in Table III. They are much alike for the five simulations, although the motions seem a little restricted in aqueous solution. The mean anisotropy in the atomic motions is comparable for all five simulations.

In Table IV the backbone conformation and its fluctuation is given in terms of ϕ-, ψ- and ω- torsional angles. The differences between the torsional angles show the same picture as in Table II; the simulations molecular dynamics in vacuo, molecular dynamics in CCl_4 and SD_{CCl_4} are much alike, the SD_{H_2O} simulation deviates more from the other ones, and the molecular dynamics simulation in aqueous solution shows the largest differences. The size of the fluctuations of the backbone torsional angles is a function of the location of the latter along the polypeptide chain. The type of solvent does not affect the size of the fluctuations much.

The sidechain torsional angles and their fluctuations are shown in Table V. The first three simulations (molecular dynamics in vacuo, molecular dynamics in CCl_4, SD_{CCl_4}) yield basically the same conformations. The two other simulations show a different conformation of the 1MeBmt sidechain. In the molecular dynamics simulation in aqueous solution this sidechain folds from its extended conformation back over the polypeptide ring, thereby exchanging an internal solute–solute hydrogen bond for the two solute-water hydrogen bonds [19]. Solute-water hydrogen bonding cannot explain the conformational change of the 1MeBmt sidechain in the SD_{H_2O} simulation.

The cyclosporin A molecule shows considerable internal hydrogen bonding (Table VI). The pattern of hydrogen bonding in the SD_{CCl_4} is the same as in the MD

Table V Sidechain torsional angles and fluctuations[a]) in CPA

Angle		*MD in vacuo*	*MD in CCl_4*	*SD_{CCl_4}*	*SD_{H_2O}*	*MD in H_2O*
1MeBmt	χ_1	−67(10)	−64(9)	−75(29)	−148(38)	−151(25)
	χ_2	163(15)	168(15)	173(16)	−132(42)	139(49)
	χ_3	−119(38)	−88(27)	−105(37)	−77(27)	−165(36)
	χ_4	167(54)	174(185)	180(49)	−139(57)	−174(49)
	χ_5	−180(8)	−180(8)	−180(8)	−179(8)	−179(8)
2Abu	χ_1	−127(50)	−100(49)	−88(45)	−131(50)	−88(42)
4MeLeu	χ_1	−78(17)	−111(43)	−90(29)	−76(11)	−75(10)
	χ_2	−77(23)	−121(49)	−82(32)	−74(12)	−72(15)
5Val	χ_1	−62(10)	−66(10)	−64(10)	−62(12)	−62(9)
6MeLeu	χ_1	−172(14)	−168(11)	−161(13)	−166(10)	−169(13)
	χ_2	−134(30)	−131(34)	−109(26)	−105(20)	−148(32)
9MeLeu	χ_1	−72(15)	−76(21)	−103(41)	−75(14)	−74(17)
	χ_2	−95(34)	−93(31)	−134(36)	−88(26)	−96(30)
10MeLeu	χ_1	−119(36)	−162(22)	−163(12)	−164(9)	−90(22)
	χ_2	−78(14)	−157(35)	−151(32)	−130(40)	−84(22)
11MeVal	χ_1	−59(9)	−58(10)	−60(11)	−59(10)	−55(10)

[a]) Root mean square fluctuation between parentheses.
Units: degrees

Table VI Hydrogen bond formation[a] in CPA

Hydrogen		*– Acceptor*		*MD in vacuo*	*MD in CCl_4*	*SD CCl_4*	*SD H_2O*	*MD in H_2O conformation*
1MeBmt	H_{γ_1}	– 1MeBmt	O	84	87	73	11	0
		– 10MeLeu	O	0	0	0	8	0
2Abu	H	– 5Val	O	61	30	31	68	23 β sheet
		– 11MeVal	O	31	58	56	40	28 γ turn
5Val	H	– 2Abu	O	88	65	72	96	72 β sheet
		– 3Sar	O	0	0	6	1	0
		– 5val	O	0	0	5[b]	3[b]	0
7Ala	H			36	49	54	20	0 γ turn
		– 5Val	O					
		– 11MeVal	O	89	49	44	67	54 β sheet
8Ala	H	– 6MeLeu	O	69	60	67	78	11 γ turn
		– 8Ala	O	2	0	0	0	0
mean[c]				42	36	37	36	17

[a] The occurrence of a hydrogen bond is given in percent of the simulation period. The hydrogen bond criterion is: the donor–hydrogen–acceptor angle θ must be larger than 90° and the hydrogen– acceptor distance must be smaller than 0.25 nm.
[b] This hydrogen bond has the unrealistic value $1\theta = 100°$; all other θ-values are larger than 127°.
[c] Mean over the 11 hydrogen bonds.

simulation in CCl_4. The molecular dynamics simulation in vacuo shows too much hydrogen bonding compared to molecular dynamics in CCl_4. Solvation of cyclosporin A in aqueous solution decreases the amount of internal hydrogen bonding considerably, due to the competition of the water molecules for hydrogen bond donors and acceptors in the solute. Therefore, it is not surprising that the SD_{H_2O} simulation shows a too high degree of internal hydrogen bonding compared to MD in H_2O.

From one-dimensional ^1H nuclear magnetic resonance experiments on a solution on cyclosporin A in $CHCl_3$ a set of 57 Nuclear Overhauser Enhancement (NOE) intensities has been obtained [33], from which a set of 57 ^1H-^1H distance constraints could be derived [20]. It would be interesting to check whether the trajectories of the two stochastic simulations reported here would satisfy these 57 different constraints. The results are displayed in Table VII, where only those proton pairs for which a

Table VII Distance constraint violations in CPA

Distance constraint[a]				r_0[a]	*Violation*[a]		
					MD in CCl_4	*SD_{CCl_4}*	*SD_{H_2O}*
1MeBmt	$C_\alpha H$	–7Ala	NH	.35	.019	.023	.002
	$C_\beta H$	–2Abu	NH	.35	.034	.041	.068
6MeLeu	$C_\gamma H$	–8Ala	NH	.35	.0	.039	.059
7Ala	NH	–8Ala	NH	.35	.016	.020	.024
8Ala	$C_\alpha H$	–9MeLeu	NCH_3	.45	.0	.013	.0
	$C_\alpha H$	–11MeVal	NCH_3	.45	.0	.0	.004
9MeLeu	NCH_3	–9MeLeu	$C_\gamma H$	.45	.0	.003	.0
	$C_\alpha H$	–10MeLeu	$C_\gamma H$	.35	.044	.029	.012
	$C_\alpha H$	–	$C_{\delta_1} H$	.45	.065	.089	.086
11MeVal	NCH_3	–11MeVal	$C_\beta H$	.30	.0	.010	.011
Sum					.178	.267	.266

[a] Distance constraint length r_0 (nm) was derived from experimental NMR data [19,33]. From the 57 distance constraints only the constraints that are violated, are shown. Violations are given in nm. Data for MD in vacuo and for MD in H_2O were not available

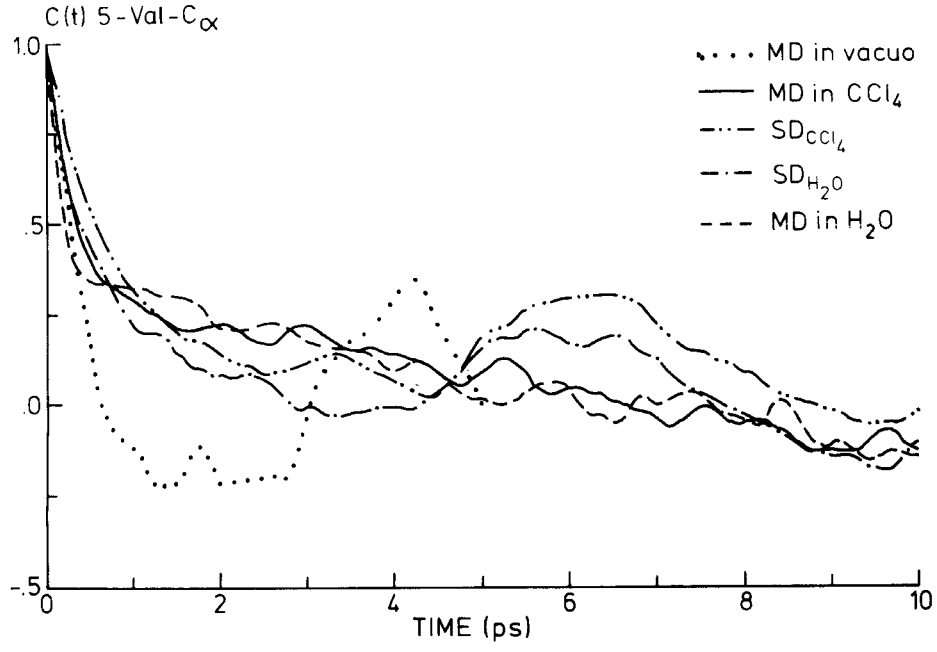

Figure 2 Atomic position fluctuation autocorrelation functions (3.1) for 5 ValC_α.

violation of the distance constraint is observed, are listed. The experimental data are well satisfied; the sum over 57 distances of the violations in the stochastic dynamics simulations only amounts to 0.27 nm, about 0.08 nm more than in the full MD simulation in CCl_4.

The dynamics of the cyclosporin A molecule in the different simulations can be compared by calculating atomic positional fluctuation autocorrelation functions

$$C(t) = (T - t)^{-1} \int_0^{T-t} [\underline{r}_i(\tau) - < \underline{r}_i >] \cdot [\underline{r}_i(\tau + t) - < \underline{r}_i >] \, d\tau \qquad (3.1)$$

where an average over the simulation trajectory is denoted by $< \ldots >$ and the length of the simulation by T. Since $C(t)$ is a single atom quantity and the length of the simulations is only 50 ps, the statistics of the atomic correlation functions (3.1) will not be very good. The atomic positional fluctuation autocorrelation functions for two backbone atoms, 5Val C_α in the β-sheet part of the molecule, and 9MeLeu C_α in the loop region, are shown in Figures 2 and 3. For 5Val C_α (Figure 2) four of the five simulations yield similar correlation functions. The molecular dynamics simulation *in vacuo* yields a correct initial decay, but fails to produce the slow decay after 0.5 ps. The correlation functions for 9MeLeu C_α (Figure 3) display different behaviour. After a quick initial decay (0.5 ps), the relaxation times are decreasing in the order: molecular dynamics in H_2O, SD_{H_2O}, molecular dynamics in CCl_4, SD_{CCl_4}. As for 5Val C_α the molecular dynamics simulation *in vacuo* deviates most from the others and shows oscillatory behaviour. The atom position fluctuation autocorrelation functions for two sidechain atoms, 4MeLeu C_{δ_1} and 9MeLeu C_{δ_1} are found in Figures 4 and 5. For 4MeLeu C_{δ_1} MD in CCl_4 (Figure 4) yields the slowest decay. Both stochastic dynam-

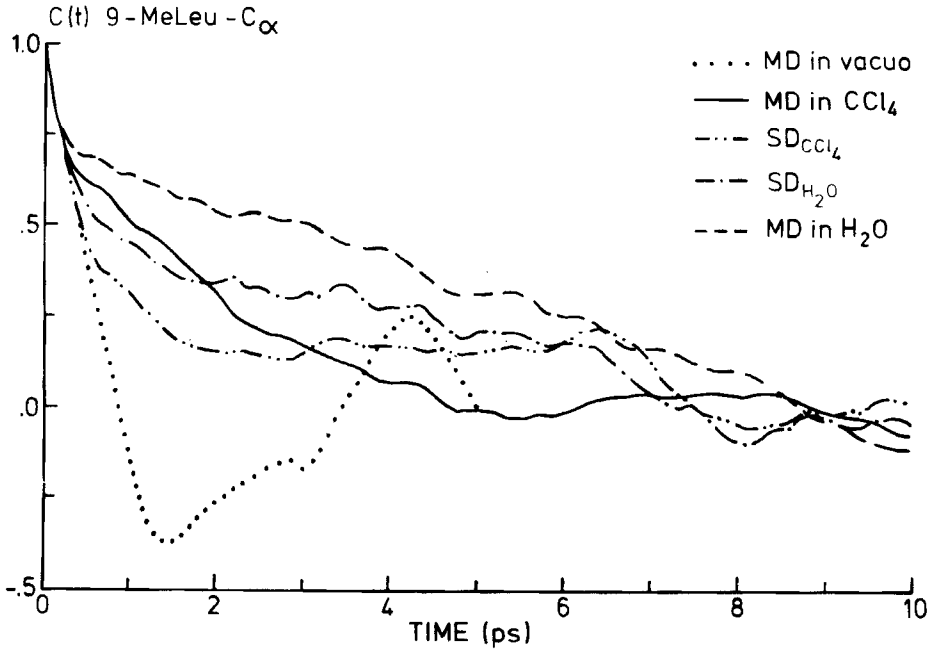

Figure 3 Atomic positional fluctuation autocorrelation functions (3.1) for 9 MeLeu C_α.

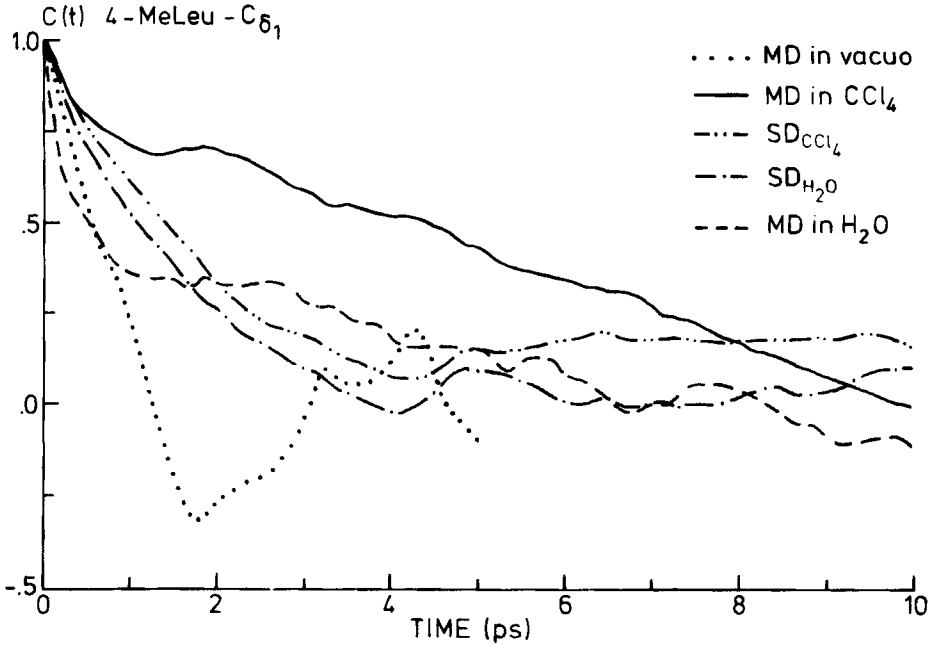

Figure 4 Atomic positional fluctuation autocorrelation functions (3.1) for 4 MeLeu C_{δ_1}.

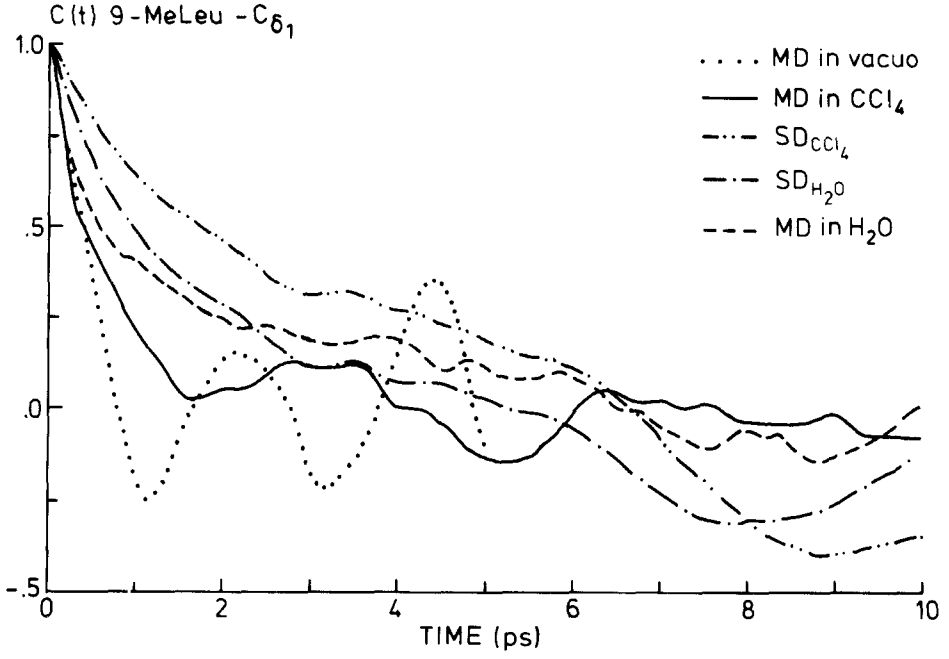

Figure 5 Atomic positional fluctuation autocorrelation functions (3.1) for 9 MeLeu C_{δ_1}.

ics simulations yield a nearly exponential decay with a shorter relaxation time. Molecular dynamics in vacuo again yields much too short a decay time. For 9MeLeu C_{δ_1} (Figure 5) the SD_{CCl_4} simulation shows the slowest decay. SD_{H_2O} and molecular dynamics in H_2O display the same pattern and molecular dynamics in CCl_4 deviates most from the other three functions. The molecular dynamics simulation *in vacuo* produces a quite different type of correlation function. In general the stochastic dynamics simulations and molecular dynamics in vacuo tend to generate smoother functions than the molecular dynamics simulations in solvent.

4. DISCUSSION

The use of stochastic dynamic simulation techniques as an approximation to full molecular dynamics simulation of a solute in the presence of solvent molecules yields a reduction of the required computing time by a factor 2 to 30, depending on the type of solvent molecule. Therefore it is worthwhile to test the quality of this approximation. For nonpolar solvents stochastic dynamics simulation without using in the interaction function an extra mean force term representing the average solvent effects on the solute, seems to be a good approximation. The properties of cyclosporin A as found in the molecular dynamics simulation in CCl_4 are well reproduced by the SD_{CCl_4} simulation. For polar solvents the omission of the mean force representing solvent effects is not justified. The hydrogen bonding properties of an aqueous solution should be expressed by the (mean) interaction function. The SD_{H_2O} simulation of cyclosporin A, in which no mean force is applied, clearly fails to reproduce the

amount of hydrogen bonding that is observed in the molecular dynamics simulation of cyclosporin A in aqueous solution. Yet, the application of stochastic dynamics using a friction coefficient derived from the experimental viscosity of water gives a better representation of full molecular dynamics in aqueous solution than a molecular dynamics simulation **in vacuo**. The application of a mean force representing the properties of an aqueous solution [11–13] is likely to bring the properties of a stochastic dynamics treatment in better agreement with those of a full molecular dynamics treatment of solute in solvent.

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