life, which in turn is due to intrauterine proximity to male fetuses.

In both mice and rats there is evidence that exposure to high concentrations of testosterone shortly after birth can result in the complete loss of both estrous cyclicity and the capacity to ovulate (*10*). This period of maximum neural sensitivity to the defeminizing action of testosterone occurs after female pups have been removed from the influence of proximal male fetuses. Exposure of 2M females to higher concentrations of testosterone than 0M females in utero does not influence their capacity to ovulate, exhibit female sex behavior, or produce and raise normal offspring in an optimum laboratory environment (*3*). But, variation in numerous characteristics that could influence reproductive success is related to prior intrauterine position. We propose that under certain ecological conditions females with a particular set of characteristics might be more likely to reproduce than other females. For example, 2M females might have a reproductive advantage over 0M females when population density is high, because they are highly aggressive toward other females but not toward males, they fiercely defend their young when lactating, and they enter puberty sooner than 0M females when housed in groups. In contrast, 0M females may be more likely to reproduce than 2M females when population density is low, because 0M females are highly preferred by males and enter puberty sooner and have shorter estrous cycles than 2M females when housed individually. Thus, it appears that 0M females are neither more nor less "normal" than are 2M females, since intrauterine proximity to male fetuses does not influence a female's basic capacity to reproduce.

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6. During day 17 of gestation numerous tissues, whose course of development is influenced by androgen, are differentiating. For instance, the rate of elongation of the perineal tissue in males as well as 2M females is at a maximum at this time [F. vom Saal, unpublished observation; R. Rugh, *The Mouse* (Burgess, Minneapolis, 1968)]. Within a single uterine horn, the percentages of female fetuses that develop next to 2, 1, or 0 male fetuses are 25, 50, and 25 percent, respectively (F. vom Saal, unpublished observation), indicating that positioning of fetuses by sex occurs at random.
7. The amniotic fluid from individual fetuses, collected on filter paper (No. 2) strips, was placed in 10 ml of acetone and incubated at 40°C for 1 hour with periodic agitation. The filter paper was removed, and the extract was dried under nitrogen. Two milliliters of sterile water was added to each sample, which was then frozen. Subsequently, 25 samples were combined prior to extraction of steroids.
8. Blood plasma was collected in 50-μl heparinized pipettes from individual decapitated fetuses. Blood collected from 25 fetuses from the same group was added to a single test tube and, after centrifugation, the serum was frozen. Blood serum and amniotic fluid were extracted with 10 volumes of a mixture of benzene and hexane (2:1) after the addition of appropriate ^{3}H-labeled steroids to monitor losses incurred during extraction and isolation. The extracts were evaporated and quantitatively transferred to a chromatographic column packed with Sephadex LH-20 (9 by 60 mm). Steroids (progesterone, testosterone, and 17β-estradiol) were eluted with cyclohexane, toluene, and methanol at a flow rate of 0.5 ml/min. Fractions were measured by procedures previously validated for mouse blood [17β-estradiol and progesterone: F. H. Bronson and C. Desjardins, *Endocrinology* **94**, 1658 (1974); and testosterone: F. H. Bronson and C. Desjardins, *ibid.* **101**, 939 (1977)].
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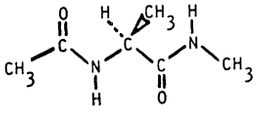
Monte Carlo Simulation of Water Behavior Around the Dipeptide *N*-Acetylalanyl-*N*-Methylamide

Abstract. *Applications of Monte Carlo and molecular dynamics computer simulation techniques indicate that they are potentially powerful tools for understanding biological systems at the molecular level. The Monte Carlo technique can be used to study the solvent structure around a small peptide and the effect of the aqueous environment on the conformational equilibria of the peptide.*

That solvent plays a crucial role in determining the structure and function of biological molecules is by now dogmatic. The study and elucidation of solvent in biological systems is, however, far from trivial and lags far behind structural studies of the corresponding biomolecules themselves (*1*, *2*). We now show how the Monte Carlo technique, a powerful computer simulation method long used in the field of statistical physics of liquids, may be used to provide insight into the effect of solvent on molecular structure and conformational equilibria. For this purpose, we have applied the Monte Carlo method to the study of the water structure around the dipeptide analog *N*-acetylalanyl-*N*-methylamide.



The dipeptide unit is the fundamental architectural unit of peptides and proteins. Because of this position, the conformation in vacuo of *N*-acetylalanyl-*N*-methylamide has been extensively studied by theoretical methods (*3*). In most cases, the effect of the solvent has either been neglected or included simply by introducing a dielectric constant. A few calculations have approximated the effect of solvent with the use of such models as the hydration shell (*4*), the "supermolecule model" (*5*, *6*) or the continuum reaction field (*7*), but none of these takes into account both the configurational fluctuations and individual molecular interactions characteristic of the solvent (*8*–*13*).

The structure of water was studied around *N*-acetylalanyl-*N'*-methylamide fixed in both the α_R ($\phi = -60$, $\psi = -50$), and C_7 ($\phi = -90$, $\psi = +90$) conformations. Here we report mainly the results of the former simulation. The dipeptide was placed in the center of a 21.73-Å cubic unit cell and 338 water molecules were packed around it to simulate infinitely dilute solution conditions. The calculated density of this system is 1.001 g ml^{-1} (for comparison, the experimental density was 1.004 g ml^{-1}) (*14*). The usual periodic boundary conditions (*15*) were used to avoid solvent-vacuum edge effects. Interactions between water molecules more than 6.2 Å apart and interactions between water molecules and the dipeptide more than 14.3 Å apart were neglected. The simulation is effectively at infinite dilution, since 14.3 Å was chosen such that no dipeptide-dipeptide interactions are included. Two simulations were carried out for each conformation in order to study the potential dependence of the results. In the first, the water-water interactions were calculated with the Rowlinson (*16*) po-

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tential, while in the second, the Stillinger and Rahman ST2 (*17*) 6–12 potential was used. The water-alanine interactions were calculated by a combination of these potentials with the 6–12 potential for peptides of Hagler *et al.* (*18*).

We generated 1.5×10^6 configurations to anneal the system, because of the arbitrary high-energy starting configuration chosen. A further 1.0×10^6 configurations were then generated by the Metropolis algorithm (*19*) to compute the properties of the system.

We have used several techniques to analyze the structure of water and its interaction with the peptide. The probability distribution of finding a water molecule in a given region of the system was calculated; an example is given in Fig. 1, which shows the distribution for a plane taken through the cell at the height of the NH_2-terminal peptide group. A peak of density is seen approximately 1.5 Å away from the NH_2-terminal amide hydrogen, indicating that a water molecule prefers to be in this position. Two similar peaks are seen about 3.0 Å from the carbonyl oxygen. This map reflects the fluctuations in position of the oxygen of the water molecule during the simulation which in this sense simulates the true behavior of a liquid, as opposed, for example, to the supermolecule or minimization approach which assumes a unique ordered structure for the water.

In order to gain more insight into the interactions and water structure at the molecular level, we have studied "snapshots" of individual configurations. An arbitrary snapshot of the system is given in Fig. 2. This shows the positions of all atoms within 3.5 Å of the dipeptide for a single configuration. Water "217" seen in the probability map of Fig. 1 has a mean position 1.6 Å from the NH_2-terminal amide hydrogen and, in Fig. 2, hydrogen bonds to it at a distance of 1.8 Å. From Fig. 1 it was noted that there were two peaks of density close to the carbonyl oxygen. These are seen to correspond to waters "244" and "289." The mean positions of these waters from the carbonyl oxygen are 3.0 Å and 3.2 Å, respectively, which suggests that they can form hydrogen bonds to it. However, in Fig. 2 only water "289" forms a weak hydrogen bond to the carbonyl oxygen at a distance of 2.3 Å. In this configuration the hydrogens of water "244" actually point away from the carbonyl oxygen and interact with waters further from the peptide (but not shown in this figure). This illustrates the importance of examining both statistically averaged distances and snapshots. Waters "297" and "209" hydrogen-bond to the carbonyl oxygen at 1.9 Å and 2.2 Å, respectively. The hydrogens of these two waters are also shown hydrogen-bonding to the oxygen of water "244", at 2.5 Å and 2.3 Å. Thus, there are three waters hydrogen-bonding to the carbonyl oxygen at this particular instant. This simulation indicates that nonstandard hydrogen bonds may be formed in a liquid environment. Such abnormal hydrogen bond interactions are frequently observed in other snapshots and have also been observed experimentally in crystal systems such as that of urea (*20*).

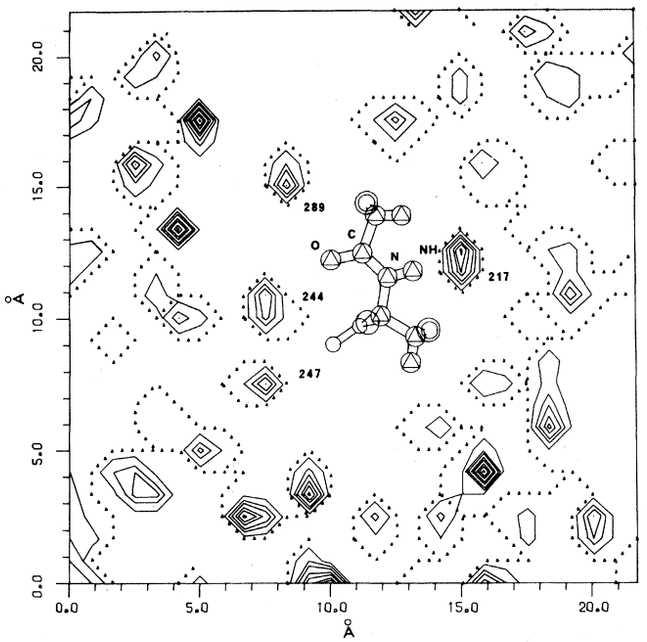

Fig. 1. Probability density map of water molecule positions for the dipeptide in the α_R conformation. The section is through the plane of the NH_2 terminal peptide group. Solid contours denote higher probabilities. Atoms within 2.0 Å of the section are shown, with atoms near the section height distinguished by triangles.

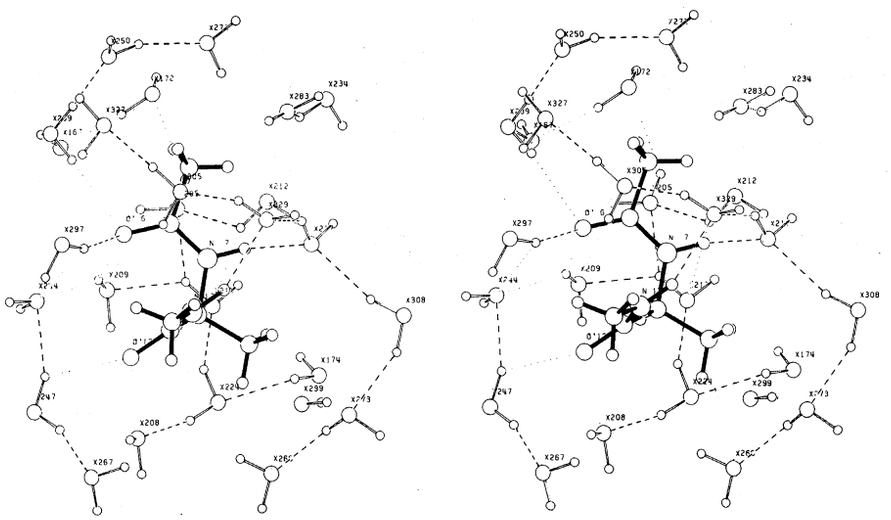

Fig. 2. Stereopicture of a single configuration showing waters within 3.5 Å of the dipeptide. Dashed lines denote hydrogen-oxygen distances of 1.5 to 2.2 Å, dotted lines denote distances of 2.2 to 2.5 Å.

To further investigate the effect of the peptide on the water structure and the range of this effect, the distributions of the water-water energy and the total interaction energy for water molecules at various distances from the dipeptide were determined. Waters near to the peptide (within ~ 3.5 Å) were found to have approximately the same total energy as in bulk water. This reflects the polar nature of the alanine dipeptide. The effect of the dipeptide was found to decrease rapidly with distance. The mean water-water energy for waters greater than ~ 4 Å were already close to the bulk value.

The converged total dipeptide-water energy for the α_R conformation is −34.1 kcal per mole of solvated dipeptide versus −28.5 kcal per mole of solvated dipeptide for the C_7, a difference between the α_R and C_7 of −5.6 kcal. The in vacuo energy difference of the two conformations computed with the 6–9 poten-

tial functions of Hagler *et al.* is +7 kcal (*21*). Ab initio molecular orbital calculations (*21, 22*) also give differences close to this value and a calculation with the use of flexible geometry gives a difference of +4.5 kcal (*21*). The dipeptide-water energy difference is thus of the same order as the energy difference in vacuo but of the opposite sign. This component of the water-peptide interaction energy would tend to stabilize the α-helical conformation relative to the C_7 in aqueous solution (*23*). This stabilization of the α_R conformation by water might be expected from the larger dipole moment of the α_R conformation due to the alignment of the two amide dipoles, 7.12 *D*, as compared to the C_7 whose dipole moment is 2.71 *D* and has an internal hydrogen bond. The results of the continuum reaction field method, which represents the interaction of the induced (macroscopic) dipole of the solvent with the solute, gives a difference between the α_R and C_7 of greater than −4 kcal (*7*). The results presented here were obtained with the Rowlinson potential. Similar results have been obtained with the ST2 potential (*23*).

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23. The total stabilization energy also includes the difference in the water-water interaction energy in the presence of the two conformers. This component takes significantly longer to converge than other averages (A. T. Hagler, D. J. Osguthorpe, B. Robson, in preparation).
24. Supported in part by grants from the Israel Academy of Sciences, NIH grant GM 24117 (A.T.H.), and an S.R.C. studentship (D.J.O.). We thank Dr. John Moult for extensive discussions and the Weizmann Institute Computer Centre and Golem B. Computer for the provision of computing facilities.
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Calcification Inside Artificial Hearts: Inhibition by Warfarin-Sodium

Abstract: *Intracavitary calcium phosphate deposits were observed in smooth, elastomeric blood pump sacs implanted in male calves for periods of 115 to 166 days. These deposits occurred predominantly on the flexing surface of the sacs. In contrast, similar pump sacs remained generally free of mineral deposits for up to 150 days in calves treated with the anticoagulant warfarin-sodium. These results implicate a vitamin K-dependent process in calcium phosphate deposition on elastomeric sacs.*

Twenty years have elapsed since the first attempts were made to use implantable blood pumps to support the circulation for prolonged periods (*1*). Although the ultimate goal of implantable blood pump programs is the development of an artificial heart, a highly desirable intermediate goal is the development of an implantable, long-term, left ventricular assist pump. Initial problems of thromboembolism and device breakage have been reduced to tolerable levels. Gradual improvements in pump design, pump fabrication, and operative techniques have enabled investigators to provide continuous left ventricular support in calves for periods exceeding 3 months (*2–4*). Moreover, three groups found that calves could survive more than 5 months after heart replacement with two pneumatically powered implanted blood pumps (*5*).

As longer-term animal studies have become possible, dystrophic calcification has been observed on the blood-contacting surfaces of the pumps removed from animals at autopsy. Calcification has been observed on pump linings fabricated of segmented polyether-type polyurethane, segmented polyurethane-polydimethyl siloxane copolymer, Dacron flock-lined polyurethane, and glutaraldehyde-treated, gelatin-coated synthetic rubber (*2, 6*). This calcification has caused stiffening, flexion failure, and perforation of the pump linings. Although this degree of calcification may be unique to the growing calf, it is now limiting the duration of studies in these animals.

In 1976, our group began to use an implantable, pneumatically powered blood pump consisting of a segmented polyurethane sac contained within a rigid plastic case (*4, 7*). The pump has been used as a left ventricular assist device or, with two such pumps, as an artificial heart, in a series of male Holstein-Friesian calves weighing 90 to 110 kg. To minimize the incidence of thromboembolism, we administered the anticoagulant warfarin-sodium and the platelet protective agents aspirin (20 mg/kg-day) and

Table 1. Sac calcification in calves not treated with warfarin-sodium.

Calf No.*	Period of continuous pumping (days)	Portion of flexing side of sac covered by gross calcification (%)†
176R	115	68.6
176L	115	59.3
108R	159	45.4
108L	159	57.1
141	166	100

*R and L refer to right or left ventricle of an artificial heart. Calf No. 141 had an implanted left ventricular assist pump. †As determined by planimetry.

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