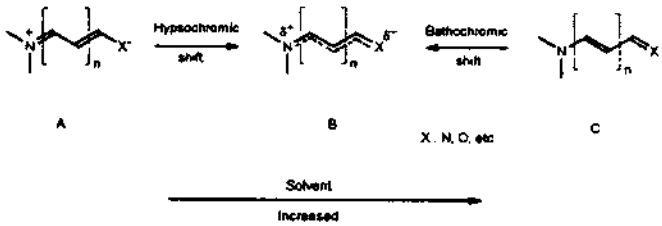
for a given dye molecule, *i.e.*, bathochromic or hypsochromic shift with increasing solvent polarity, depends largely on the change in polar characteristics between ground and exited states.[3a] For a weakly polar molecule, with low polarity in the ground state and increased polarity in the excited state, a bathochromic shift results. Conversely, for a highly polar molecule in the ground state and reduced polarity in the excited state, a hypsochromic shift is observed. In principle, a merocyanine dye could exhibit either a bathochromic or a hypsochromic shift since the ground state could have an electronic configuration corresponding largely to the nonpolar quinoid IIb, or to the dipolar, zwitterionic form IIa, or an inbetween character. In general terms, we may represent the ground state of the merocyanines as a polyene-like structure A and the excited state as a plyamethine-like structure B, a situation which will result in a hypsochromic shift. Conversely, a bathochromic shift would have results if the ground state had the quinoid structure C and the excited state the dipolar structure B.



The observation of a hypsochromic shift (negative solvatochromism) in the present system is hence indicative of a highly polar ground state, *i.e.*, approaching the configuration of the zwitterionic form IIa, and an excited state with decreased polarity. The branched linear plot with zero slope obtained in the less polar-solvent region (E_T<40) for the compound **1** and **2** may indicate and inbetween character in the ground state.

This doubly branched solvatochromic behavior of 5-chlorinated 1,3,3-spiro(2H-1-benzo-pyran-2,2'-indolines) (**1-3**) is quiet in coincidence with the recent our report[4] of the solvatokinetic behavior of those compounds **1-3**. Doubly branched solvatokinetics is certainly indicative of a structural change of the ring opened merocyanine, between polyene-like ionic structure IIa and the quinonoidal structure IIb in the ground state and hence alter the reaction mechanism for the spiro-ring formation.

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Macromolecular Modeling and NMR Distance Geometry Refinement: Conotoxin G1 in Solvent

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Conotoxin is a neurotoxin peptide of fish-hunting cone snails.[1] The structure of conotoxin-G1 is NH_2-Glu-Cys-Cys-Asn-Pro-Ala-Cys-Gly-Arg-His-Tyr-Ser-Cys-NH_2 with disulfide bridges of Cys2-Cys7 and Cys3-Cys13. In spite of the disulfide bridge, the molecule is rather flexible in solution.[1] To study the structure, Pardi *et al.*[2] reported 50 NOE distance geometries from 2-D NMR experiment. The 2-D NMR experiment has been a very useful method to study flexible molecular structures.[3] But, the NMR data can provide only a piece of information to analyze the overall structures. Nevertheless, the information is very useful to understand protein folding. To utilize such information, we have performed various simulations for conotoxin-G1 with new methodology, comparing the theoretical result with the NOE data.

To devise a realistic effective method to include solvent effects, we used water clusters that are pseudo-bound to charged residues with weak interaction potentials. The water cluster reduce direct interactions between charged residues, simulating solvent effects.[4] Therefore, the water-cluster method can be utilized to find the most statistically significant conformations of macromolecules in solution and to refine NMR distance geometries.

In our molecular dynamics simulation, we used Kollman's interaction potentials.[5] But, the dielectric constant for the coulombic interactions was chosen as $\varepsilon = 1/[1-(r/r_c)^2]^3$, so that the long-range coulomb potential was smoothed near the cut-off region. Here, r, is the intersite distance, and r_c is the cut-off radius chosen as 8 Å. This method removes

abrupt potential discontinuities, and treats solvent effects effectively with small number of water molecules, Further, it generally provides more reasonable results[4] than the commonly used $\varepsilon=r$.

To find the low-lying energy conformations of conotoxin-G1, a number of different initial structures were generated with artificially randomized constraints at extremely high temperatures. For small molecules, the global minimum can be located with the minimization procedure, which can find the same lowest-energy sturucture from many a different initial conformers.[5] However, for macromolecular systems, one can hardly expect to find the same lowest energy structures due to enormous multiminima. Utilizing ensemble values at a certain temperature (such as 300 K), molecular dynamics can scan over numerous neighboring local minima with low activation barriers as one equivalent structure. In this way, annealing-quenching processes generate several inequivalent structures from many different initial structures.

In our simulation, the constraints for the distance geometries were given as $V=K_\beta[1-(a^*/r)^\beta]^2$, where V, K_β, and a^* are constrainst energy, constraint potential parameter, and NOE intersite distance. The potential costant K_β can be considered as the energy that the constrainst is released. The harmonic potential type corresponds to the case when $\beta=-1$, while the 6-12 potential type (related to NOE distance geometry refinements) corresponds to $\beta=6$. At the beginning stage of the simulations, β of -1 is used to obtain the correct constraint values from the starting geometries. But, near equilibrium stage, positive β values are used to stress short-range-distance constraint. Since NOE experimental data have generally somewhat large error deviations, we set $a^*=a_{min}$ for $r<a_{min}$, and $a^*=a_{max}$ for $r>a_{max}$, and $V=0$ for $a_{min}<r^*<a_{max}$.

Since NOE intensity is proportional to r^{-6}, NOE deviations are defined by $\text{r6dev}^*=\{\Sigma(1/r^6-1/a^{*6})^2/N\}^{1/12}$, and $\text{r6dev}=\{\Sigma(1/r^6-1/a^6)^2/N\}^{1/12}$, when N is the number of distance geometries. Mathematically the least NOE deviations should be chosen as $\beta=6$ instead of $\beta=-1$ (of the harmonic constraint type). This point is extremely important because it is not appropriate to optimize $\Sigma(r-a)^2$ for flexible molecules or multi-conformes.

For conotoxin-G1, two hydrogen atoms (H_a, H_b) attached to the same heavy atom often show different chemical shifts due to disulfide bridges. It is sometimes unclear whether a certain chemical shift is due to H_a or H_b. Further, before the equilibrium in simulation (in particular, when the initial structure was started as a stretched linear structure), it is difficult to have the correct assignments for H_a and H_b which change with structural change. The difficulty is resolved by using $V=V_aV_b/(V_a+V_b)$, where V_a and V_b are the constraint potentials for the intersite distances from the considered hydrogen atom to H_a and H_b, respectively. Then, the correct assignments are automatically obtained, even if the assignments are continuously changed.

If there are degenerate chemical shifts, the effective NOE distance[7] is used with $r=(1/r_a^6+1/r_b^6+\cdots)^{-1/6}$. This effect was explicitly implemented in computer program for all possible cases (such as methyl hydrogens in glycin, and δ and ε carbon hydrogens for tyrosine ring flipping). More complex schemes were also used when H_a and H_b neighbored to more than one hydrogen atom have a few different types of chemicals shifts.

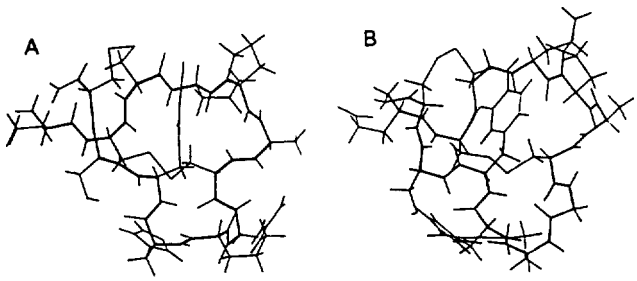

Figure 1. Conotoxin G1 Structures from Simulations: (a) MDW 4: A and (b) MDW4: B.

Table 1. NOE Distance Deviations from Simulations

	Energy	rdev6*	rdev6	rdev*	rdev
MDC4: A	−677	.54	.12	.55	.14
MDC4: B	−670	.79	.43	.87	.49
MDC0: A	−683	.82	.43	.89	.53
MDC0: B	−687	1.3	.88	1.5	1.1
MDC0: A+B		.61	.25	1.1	.66
MDW4: A	−3856	.58	.17	.61	.22
MDW4: B	−3845	.73	.34	.80	.39
MDW0: A	−3872	.70	.28	.71	.31
MDW0: B	−3855	.88	.50	.89	.53
MDW0: A+B		.56	.16	.74	.28

Energies and distances are in kcal/mol and Å, respectively.

Using the molecular dynamics simulations with pseudo-bound water clusters (MDC0), several low-lying energy conformations were obtained for conotoxin-G1. Among these, two conformers (A and B) have somewhat lower energies, and satisfies most of the NOE distance geometries. Based on the two conformers, molecular dynamics simulations of conotoxin in bulk-water (MDW0) were performed with 320 water molecules. Then, weak NOE constrainst of $K_6=4$ kcal/mol was added to both structures from MDC0 and MDW0. The two new simulation results are denoted as MDC 4 and MDW4, respectively. Figure shows two low-lying energy conformers (A and B) in water which were refined with the NOE constraints of $K_6=4$. Table lists r6dev* and r6dev as well as rdev* and rdev which are the root mean square deviations of $r-a^*$ and $r-a$, respectively. Conformer A can be considered as the most stable with the least NOE deviation. But, since both conformers of A and B are energetically almost equal, we consider the statically equally weighted mixture. The mixture has smaller r6dev and r6dev* than each conformer, and explains the unresolved or uncertain NOE data more properly without extra peaks. Nevertheless, conformer A (MDW4) with NOE refinements alone agrees well with the NOE data. Since the potential used is not reliable enough to derive our conclusion, further study is in progress to better decide whether the protein comprises only one conformer (A) or the mixture of a few conformers. However, this communication does not stress the possibility of multi-conformers in conotoxin, but stress that dynamical structures

and multiconformers can play important roles in interpreting the NMR data. Consequently, for the study of molecules having multiconformers and high flexibility the sum of r6dev shoud be minimized instead of rdev.

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