CRYSTAL AND MOLECULAR CONFORMATION OF THE FLUORESCENT PROBE, 8-ANILINO-1-NAPHTHALENESULFONIC ACID (ANS).

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Summary

The crystal and molecular structure of the fluorescent probe 8-anilino-1-naphthalenesulfonic acid (ANS) has been determined as the ammonium monohydrate with two conformationally distinct molecules in the triclinic $P\bar{1}$ lattice. The angles between the aromatic rings and the C-N-C plane are -9/22° and 112/22° respectively. There is an N-H...O intramolecular hydrogen bond in each molecule indicating that hydrogen bond formation is not dependent on the anilino geometry. There are also short intramolecular H...H contacts involving the hydrogens which have anomalous proton shifts shown in a recent NMR study.

The enhancement of fluorescent properties of 8-anilino-1-naphthalenesulfonic acid (ANS) as a result of noncovalent interaction with a protein has made it useful as a probe of hydrophobic sites on proteins. As such, it has been used to explore the binding sites of many substrates, enzymes, membranes and proteins.[1-4] Studies also show that ANS will displace the thyroid hormones T_3 and T_4 from their binding sites on the carrier proteins thyroxine binding globulin and serum albumin.[5-7] Since ANS binds to specific regions of these proteins, it has been used to elucidate the conformational changes affecting specific binding sites as well as the number and affinities of these sites.

The crystal structure of ANS was undertaken in order to compare its conformation with that of the thyroid hormones T_3 and T_4 and to determine its conformational preference in light of the current mechanisms proposed for fluorescence.

Methods

Crystals of 8-anilino-1-naphthalenesulfonic acid (ANS), purchased from Eastman Kodak Chemicals, were grown at room temperature from an aqueous solution. The cell parameters for the triclinic $P\bar{1}$ lattice, $Z = 2$ are a = 11.0117(9), b = 13.935(2), c = 10.4519(8)Å, α = 99.811(6), β = 95.423(6), and γ = 100.142(8)°. Intensities for 6333 (5892 observed) independent reflections with $\theta<75°$ were measured on a Nonius CAD-4 diffractometer using CuKα radiation.

The structure was solved by direct methods using MULTAN.[8] All non-hydrogen atoms for the two molecules of ANS were located in the resulting E-maps from MULTAN. Acceptable atomic positions for all hydrogens were located from three-dimensional Fourier difference maps. Both the positional and isotropic thermal parameters for the hydrogen atoms were refined. The structure was refined by full-matrix least-squares. The final R-index is 0.068.

As illustrated in Figure 1, the conformations of the two molecules of ANS differ significantly from one another. The C-N-C angles are 123.4° and 127.3° in ANS(1) and ANS(2) respectively. The planes of the phenyl and naphthalene rings make angles of 112/22° and -9/22° with the C-N-C planes in ANS(1) and ANS(2) respectively. However, the dihedral angles between the phenyl and naphthalene rings are 63° and 53° respectively. The anilino nitrogen in ANS(1) has a slightly distorted tetrahedral geometry as indicated by the sum of the valency angles of 335.7°, whereas in ANS(2) this configuration is slightly distorted from trigonal geometry where the sum of the valency angles is 357.6°.

The overall geometry of ANS(1) is similar to that observed for the thyroid hormones T_3 and T_4 [9,10] and that of ANS(2) resembles the fluorescent probe, 2-*p*-toluidinyl-6-naphthalenesulfonic acid (TNS).[11]

Discussion

While the mechanism of fluorescent enhancement in proteins is not clearly

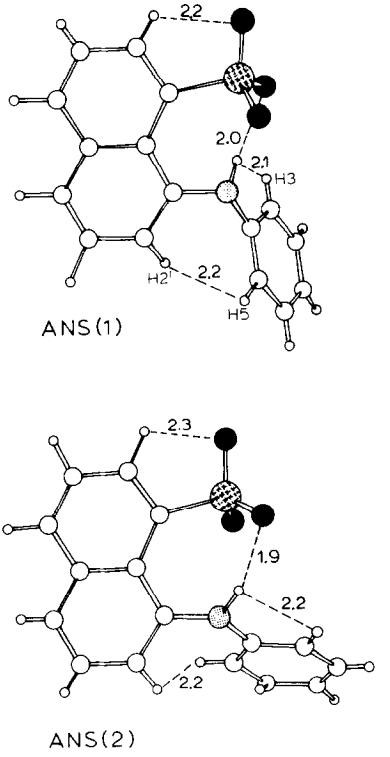

FIGURE 1. Molecular conformation of the two conformers of ANS showing intramolecular hydrogen bonds and close H...H contacts.

understood, it has been suggested that the relative orientation of the two rings in the probes may influence their fluorescence characteristics.[2,12,13] The results of a recent NMR study on ANS [13] suggest that the aromatic rings of ANS are more nearly coplanar in alcohol than in water and that the anilino hydrogen participates in a strong interaction with the sulfonate group in alcohol. This study also shows that there is an anomalous behavior in the shifts of the H3, H5 and H2' proton signals in both solvents which is attributed to abnormal deshielding due to ring currents in both aromatic systems. Finally, it indicates that solvents which favor intramolecular hydrogen bond formation will favor a conformation which has the rings nearly coplanar.

The results of this structural study show that despite the gross conformational differences in the two ANS molecules there are (1) intramolecular

N-H...O hydrogen bonds in both conformers and, (2) short intramolecular H...H contacts involving the H3, H5 and H2' protons in both conformers. The presence of these features in both molecules suggests that they are independent of the anilino geometry and may explain the anomalous NMR behavior of the proton signals. Since ANS(2) is more coplanar than ANS(1), the ANS(2) conformation may be the one adopted by the molecule in alcoholic solutions while ANS(1) may represent the conformation observed in water. Also, since these two different conformations are observed under identical crystallization conditions, it is suggested that a nearly coplanar geometry of the rings is not necessary to favor intramolecular hydrogen bond formation.

It has also been reported recently that in the protein structure of horse liver alcohol dehydrogenase there are two binding sites for ANS. The preliminary results show that the ANS molecules in these sites are bound in two different conformations,[14] perhaps the conformations observed here.

If the relative orientation of the two rings has a significant influence upon the mechanism of fluorescence,[12-14] then the fluorescent properties of the two conformers observed here and in the protein receptor sites are probably different. The observation of two different non-coplanar conformers in these crystals as well as in the protein receptor sites suggest that a coplanar conformation of the two rings may not be essential to fluorescence.

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