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# NMR STUDY OF 1:1 COMPLEXES BETWEEN DIVALENT SULFUR AND AROMATIC COMPOUNDS: A MODEL FOR INTERACTIONS IN GLOBULAR PROTEINS

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*Summary: The existence of 1:1 complexes between various divalent sulfur and aromatic compounds in* $CCl_4$ *solution is demonstrated by the method of Job. By making measurements at different temperatures we find that the heat of complex formation is close to 1 kcal/mol. We propose that this interaction, which is stronger than that due to van der Waals' forces alone, be included in attempts to calculate the energy of native proteins or to predict the folding of polypeptide chains.*

## Introduction

In the folding of globular proteins, the side-chains of the various amino acids are brought into van der Waals' contact. For the most part, unless the side-chains can form H-bonds or ionic links, these contacts are non-specific. Recently, however, the existence of chains of alternating sulfur-containing and aromatic groups within globular proteins has been pointed out[1]. The presence of these chains raises the possibility that there may exist some more specific attraction between sulfur and aromatic groups. In order to investigate this possibility, we undertook the experiments here described. Small molecules, whose structures are similar to the side-chains of the sulfur-containing and aromatic amino acids, were chosen for study. As we will demonstrate, 1:1 complexes, whose heats of formation approach 1 kcal/mol, are formed in carbon tetrachloride solutions containing pairs of such small molecules.

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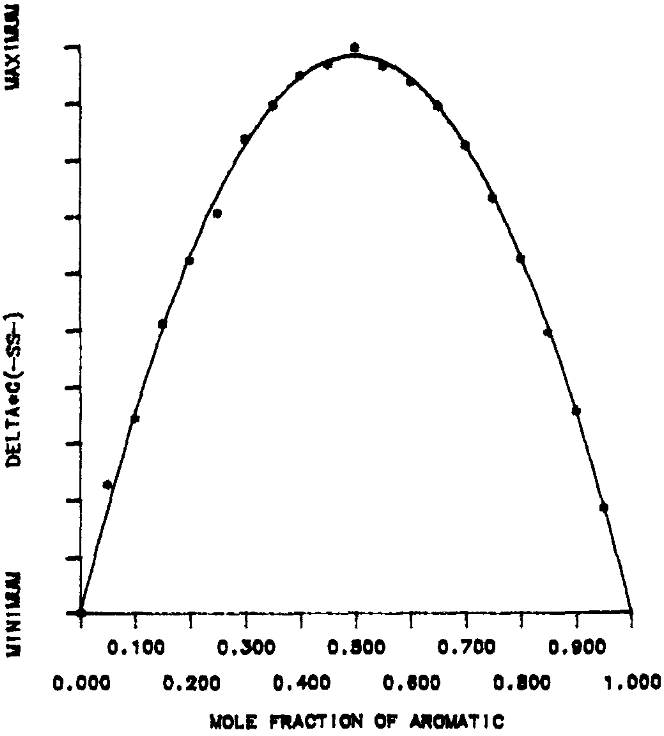
## Experiments and Results

It is well known[2] that proton chemical shifts for polar molecules change, often dramatically, if benzene or toluene is used as solvent in place of $CHCl_3$ or $CCl_4$. Such shifts are known as "aromatic solvent induced shifts" or ASIS. There has, however, been considerable debate[3] as to whether these effects are the result of direct solute-solvent association or whether they are a consequence of the structure of the solvent cavity in which the solute exists. This latter model corresponds to no specific interaction between



CURVE CALCULATED FOR 3.7 M, K(2)=0.025
POINTS ARE DATA FOR 3.7M

Fig. 1. Job plot for the system dimethyl disulfide (S)--1-methylnaphthalene (Ar) in $CCl_4$, total concentration = 3.7 M. The ordinate gives the product of the observed shift of the methyl protons of S and the concentration of S. The chemical shift of the 1:1 complex was taken to be 100 Hz; the equilibrium constant, $K_2$, was 0.025. The maximum in the computed curve comes at [Ar] = 0.50.

Experimental points are asterisked; computed curve is the solid line. Measurements were made on a Brucker WP-200 NMR Spectrometer with samples at 26°C. An essentially identical set of points was obtained for the same system at a total concentration of 1.1 M.

solvent and solute, whereas the former implies the existence of a complex of well defined stoichiometry and stereochemistry. We have now applied the method of Job[4] to investigate the formation of complexes between dimethyl disulfide (S) and 1-methylnaphthalene (Ar), a system which serves as a model for sulfur-aromatic interactions in proteins. Figure 1 shows a typical Job plot, the position of whose maximum is clearly consistent with the formation of a 1:1 complex. Both the position of the maximum and the shape of the Job plot, however, can be influenced by other equilibria. We have therefore simulated on the computer Job plots for the following set of equilibria:

$$2Ar \underset{}{\overset{K_1}{\rightleftharpoons}} Ar_2 \tag{1}$$

$$Ar + S \underset{}{\overset{K_2}{\rightleftharpoons}} Ar{\cdot}S \tag{2}$$

$$2Ar + S \underset{}{\overset{K_3}{\rightleftharpoons}} Ar_2S \tag{3}$$

Our studies show that inclusion of the equilibrium (3) causes a skewing of the Job plot, as well as a shift in its maximum and hence of the apparent stoichiometry of the complex. Figure 2 shows that this effect can be seen even for $K_2/K_3 = 20$. The deviations caused by the introduction of equilibrium (3) can be removed by including self-association of the aromatic component, i.e. equilibrium (1). However, to obtain a fit as good as that shown in Fig. 1, $K_1/K_2$ must be approximately 0.5, and proportionately larger as the value of $K_2/K_3$ is decreased. Thus, any substantial amount of equilibrium (3) requires stronger self-association of the aromatic component than is consistent with the known physical properties (e.g., vapor pressure) of aromatic liquids. Furthermore, while a good fit may be obtained in this way for one value of the total concentration [Ar] + [S] of the two components, the set of three equilibrium constants used no longer produces the best fit at higher concentrations. Thus, the best fit to data obtained at both 1.1 M and 3.7 M total concentrations requires the model in which only the 1:1 complex prevails. We therefore believe that the observed changes in chemical shifts are essentially a result

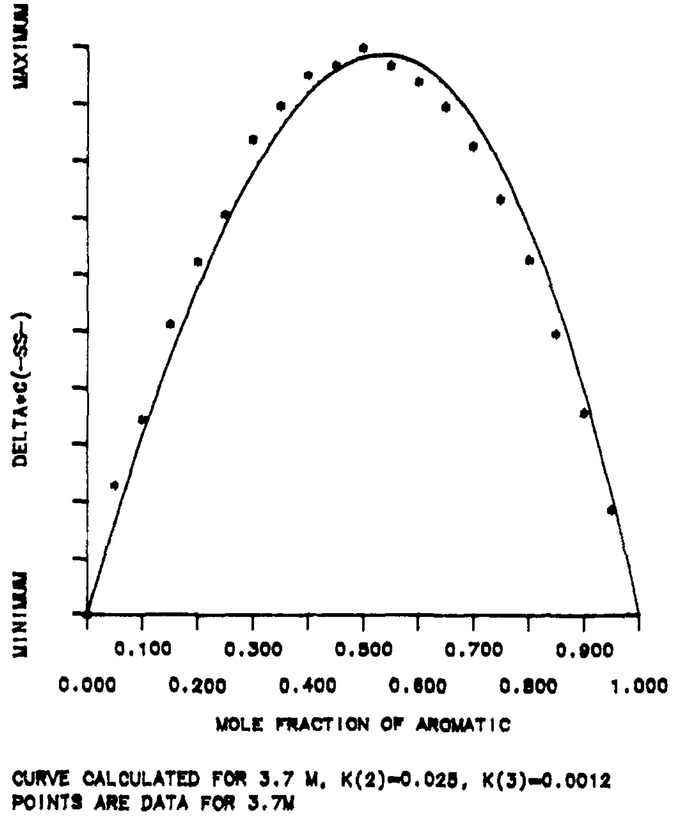


Fig. 2. Job plot for the same system and data as Fig. 1, but compared with a curve computed for two equilibria, with constants $K_2$ = 0.025 and $K_3$ = 0.0012. The maximum in the computed curve comes at [Ar] = 0.54.

of 1:1 complex formation with only minor contributions from higher clusters. Results for other systems studied are summarized in Table 1.

In order to compute the heat of complex formation, it is necessary to estimate the absolute value of $K_2$. This constant is related to the chemical shift which we observe by equation (4)

$$\delta_{obs} = (K_2 \delta_{Ar \cdot S}[Ar])/(1 + K_2[Ar] \qquad (4)$$

where $\delta_{Ar \cdot S}$ is the chemical shift of pure complex.

Attempts to determine the absolute value of $K_2$ by nonlinear-least squares fitting of the data to equation (4) were unsuccessful. Only in the case of dimethyl disulfide (0.1 M) 1-methylnaphthalene (0.2-1.8 M) was convergence obtained, and even then $K_2$ (0.06 $mol^{-1}$) and $\delta_{Ar \cdot S}$ (2 ppm) were very

Table 1

Chemical shifts and heats of complex formation for various sulfur-aromatic systems

| System | | | $\Delta\delta_{AB}$[a] | [X][b] | $\Delta H$[c] |
|---|---|---|---|---|---|
| Dimethyl sulfide | - | Benzene | 3.5 | 0.5 | |
| Dimethyl sulfide | - | Toluene | 6.9 | 0.5 | |
| Dimethyl sulfide | - | *p*-Cresol | 3.9 | 0.5 | |
| Dimethyl sulfide | - | MENAP[d] | 12.5 | 0.52 | |
| Dimethyl disulfide | - | Benzene | 5.4 | 0.52 | |
| Dimethyl disulfide | - | MENAP | 18.1 | 0.52 | |
| Cyclopentane | - | Benzene | -0.7 | *e* | |
| Tetrahydrofuran (α) | - | Benzene | -0.7 | | |
| Tetrahydrofuran (β) | - | Benzene | 3.8 | 0.5 | |
| Tetrahydrothiophene (α) | - | Benzene | 2.4 | | |
| Tetrahydrothiophene (β) | - | Benzene | 5.1 | 0.5 | |
| Dimethyl sulfide | - | MENAP[f] | 14.8 | | -0.79 |
| Dimethyl disulfide | - | MENAP[f] | 21.7 | | -0.96 |
| Tetrahydrofuran (α) | - | MENAP[f] | 5.0 | | |
| Tetrahydrofuran (β) | - | MENAP[f] | 8.4 | | -0.91 |

*a* shift in going from 1.7 M S and 0.3 M Ar to 0.3 M S and 1.7 M Ar

*b* mol fraction corresponding to maximum value of $\delta_S C_S$ in Job plot

*c* kcal mol$^{-1}$.

*d* 1-methylnaphthalene

*e* exhibited no maximum

*f* shift in 0.1 M S caused by the addition of 1.8 M MENAP

strongly correlated. Accordingly, we examined only the concentration range (0.0-1.0 M) in 1-methylnaphthalene for which the observed shifts were a linear function of (Ar). This allowed the evaluation of the product of $\delta_{Ar\cdot S}\cdot K_2$ from sets of data obtained at various temperatures (-26° to 29°C). ΔH for equilibrium (2) was then calculated from the slope of plots of $\ln(\delta_{Ar\cdot S}\cdot K_2)$ against

1/RT, assuming $\delta_{Ar \cdot S}$ to be temperature-invariant. The values for ΔH thus obtained are included in Table 1. They approach 1 kcal/mol.

The direction and magnitude of the shifts for the α- and β-protons of tetrahydrofuran and tetrahydrothiophene provide a picture of the 1:1 complex in which the hetero-atom is situated above the edge of the aromatic ring with its electron lone-pairs directed away from the ring. This places the β-protons nearer the center of the aromatic ring where the magnetic shielding effect of the π-electrons is greatest.

## Discussion

The native folding of a polypeptide chain into a three-dimensional globular protein is the result of a delicate balance among several opposing tendencies. In attempts to predict folding, the presence of a specific term whose magnitude may be as high as 1 kcal/mol cannot be neglected. While not large in itself, this term is 3-5 times greater than current estimates of the van der Waals' interaction between sulfur and aromatic side-chains[5]. The choice of 1-methylnaphthalene as the aromatic component provides an upper bound for this type of interaction which is only likely to be reached in proteins for sulfur-tryptophan pairs, and then only when these groups are optimally oriented. On the other hand, proteins also contain groups with positive electric charges. We have not attempted to model this situation, but have recently shown that the presence of these groups is strongly correlated with the occurrence within the protein of sulfur-aromatic interactions[6].

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