

**Abstract**—Heteroassociation of an anthracycline antibiotic Daunomycin (DAU) and phenanthridine dye Propidium iodide (PI) in aqueous solution was studied by $^1$H NMR spectroscopy. The complex PI–DAU is stabilized mainly by dispersion van der Waals interactions and hydrogen bond between the 3(8)-amino group of the dye and 9-acetyl group of DAU. This conclusion follows from comparison of parameters of DAU–PI heteroassociation and complex formation of DAU with aromatic dyes, Proflavine and Ethidium bromide, under the same conditions.

Studies of heteroassociation of biologically active aromatic molecules are important from both theoretical and practical viewpoints. First, they provide information on the nature of physicochemical interactions which affect the affinity of aromatic molecules in solution and depend on structural features of the chromophore and side chains of the aromatic ligand. Second, from the medicobiological viewpoint, heterocomplexes formed by aromatic compounds and their concurrent binding to receptors could influence the solubility and efficiency of antibiotics. For example, caffeine and nicotinamide (vitamin PP) increase the solubility of a series of antibiotics in aqueous solution [1, 2]. Aromatic compounds isolated from food raw materials, such as polyphenols and methylxanthines, can act as regulators of pharmacological activity of antibiotics and DNA protectors from complex formation with aromatic mutagens [3–6]. This problem also includes some aspects of using antibiotics in combination with other drugs in pharmacotherapy [7, 8]. Thus studies of heteroassociation of aromatic molecules are related both to the solubility and efficiency of antibiotics and to the diet in chemotherapy [9, 10].

Various heteroassociation models have been proposed in the recent years for aromatic molecules [4, 5, 11–15]. However, most of these are characterized by fairly severe limitations which restrict their practical application (for details, see [16, 17]). As a rule, the proposed models [4, 5, 11–15] do not consider the possibility for formation of multidimensional aggregates of any size via both self-association and heteroassociation. Also, they provide no analytical expressions which could be convenient for experimental data processing. In order to reliably determine NMR parameters (at a sufficiently high signal-to-noise ratio) it is necessary to perform measurements at relatively large concentrations of aromatic compounds (about several mmol/l). Therefore, models for analysis of NMR data should take into account formation of not only dimeric but also associates of higher order via self- and heteroassociation of molecules [18].

We recently developed a statistical–thermodynamic heteroassociation model for analyzing NMR data of aromatic molecules in a mixed solution [16, 17]. According to this model, molecules form infinite-dimensional aggregates via both self-association and heteroassociation, and there are no limitations for the equilibrium self-association constants. In the present study we applied the developed model [16, 17] to the analysis of heteroassociation of an anthracycline antibiotic Daunomycin (DAU), which exhibits antitumor activity, and phenanthridine dye Propidium iodide (PI), which possesses pronounced mutagenic properties, using one- and two-dimensional $^1$H NMR spectroscopy (500 MHz). The structures of DAU and

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molecule of Propidium iodide contains a longer aminoaalkyl side chain and has an additional positive charge, which strongly influence its self-association parameters. The self-association constant of PI ($K_{PI} = 46$) is by a factor of ~5 smaller than that of EB ($K_{EB} = 305$) at 298 K. Comparison of the heteroassociation parameters of the phenanthridine dyes with those of Daunomycin led us to draw some conclusions on the nature of physicochemical interactions and the role of side chains in the aromatic molecules in the formation of heterocomplexes in solution.

The structural and thermodynamic parameters for complex formation of Daunomycin with Propidium iodide were determined, as it was done previously for the system Ethidium bromide–Daunomycin [19], by analysis of proton chemical shifts of the dye and antibiotic in solution at various concentrations and temperature. Figure 2 shows the plots of proton chemical shifts of PI and DAU at 303 K and versus temperature. While varying the concentration of DAU, the concentration of PI was maintained constant ($P_0 = 0.79$ mM). The self-association equilibrium constant of DAU is more than an order of magnitude greater than the self-association constant of PI ($K_{PI} = 46$). $K_{DAU} = 580$ l/mol [21] at 303 K; therefore, the concentration of DAU has a stronger effect on the equilibrium distribution of aggregates than does the concentration of the dye.

As previously [17, 19], the experimental data were analyzed following the general molecular heteroassociation model which assumes the existence of a dynamic equilibrium in solution. Here, the equilibrium involves infinitely dimensional self-association and heteroassociation with formation of different complexes (Scheme 1):

Scheme 1.

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\begin{align*}
A_1 + A_j & \rightleftharpoons K_A A_{j+1} \\
(A) \\

P_1 + P_f & \rightleftharpoons K_P P_{f+1} \\
(B) \\

A_j + P_f & \rightleftharpoons K A_j P_f \\
(C) \\

P_i A_j + P_f & \rightleftharpoons K P_i A_j P_f \\
(D) \\

A_k + P_i A_j & \rightleftharpoons K A_k P_i A_j \\
(E)
\end{align*}
\]

Here, $A_i$ and $P_j$ are, respectively, monomeric DAU and PI; $A_0$, $A_k$, $P_i$, and $P_i$ are aggregates containing