Intermolecular Bonding of the Antibiotic Diumycin

JOEL KIRSCHBAUM, WILLIAM A. SLUSARCHYK, and FRANK L. WEISENBORN

Abstract ☐ The closely related antibiotics diumycin A and B, with monomeric molecular weights of approximately 1800 daltons in ethanol, aggregate in aqueous buffers to form particles with a molecular weight (mol. wt.) of at least 32,000 daltons. The aggregate of diumycin is essentially unaffected by esterification of the acid, acetylation of hydroxyl groups, high ionic strength buffer, or variations in pH from 2.2 to 12.4. These results indicate that salt linkages and hydrogen bonds contribute only slightly to stabilize the aggregate. The aggregate may be disrupted by: (*a*) the addition of such hydrophobic bond-breaking agents as buffered aqueous solutions of guanidinium chloride, urea, or formamide; (*b*) the hydrolytic loss of a lipid side chain (mol. wt. about 400 daltons); or (*c*) the addition of alcohols. The ability of an alcohol to disrupt the aggregate increases with its hydrocarbon content. From these data, it is concluded that lipid–lipid hydrophobic interactions are responsible for the self-association of diumycin. The aggregate is spherical with a mantle of hydrophilic sugars, including glucose and glucosamine, surrounding a tangle of lipid side chains.

Keyphrases ☐ Diumycin—intermolecular bonding ☐ Aggregates, diumycin A, B—ethanolic aqueous buffers ☐ Hydrogen, lipid–lipid hydrophobic bonding—diumycin ☐ Sedimentation coefficients —diumycin ☐ Molecular weight—diumycin

The diumycins are a family of closely related antibiotics isolated from *Streptomyces umbrinus*, with formula molecular weights between 1700 and 2100 daltons, as determined by elemental analysis, including phosphorus content (1).

This paper concerns the ability of diumycin to aggregate in aqueous solvents, an ability shared by many natural products (2–5), and the source of intermolecular binding energy for such aggregation.

EXPERIMENTAL

Sources of Diumycin and Diumycin Derivatives—Diumycin A and B were isolated and characterized by Meyers *et al.* (1). Weisenborn *et al.* (6) prepared the derivatives of diumycin. Hydrolysis of purified diumycin, with 1 *M* HCl at 100° for 30 min., yields a 358-dalton lipid (6).

Sedimentation Coefficients—Sedimentation coefficients were determined with the aid of a Spinco model E analytical ultracentrifuge, using a calibrated temperature-control unit. Viscosity was measured with the aid of capillary and Zimm viscometers (7). One-milliliter pycnometers were used for density measurements at 20 ± 0.002°. Viscosity and density corrections were also taken from the data of Kawahara and Tanford (8) for adjusting the sedimentation coefficients to standard conditions (9), which are the viscosity and density of water at 20°.

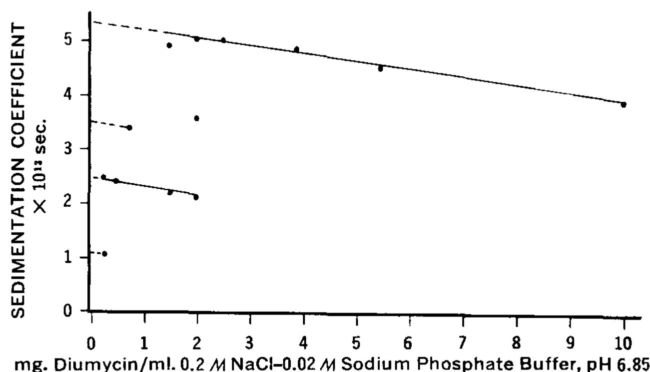


Figure 1—*Dependence of the sedimentation coefficient of diumycin A on its concentration in 0.2* M *NaCl–0.02* M *sodium phosphate buffer, pH 6.85.*

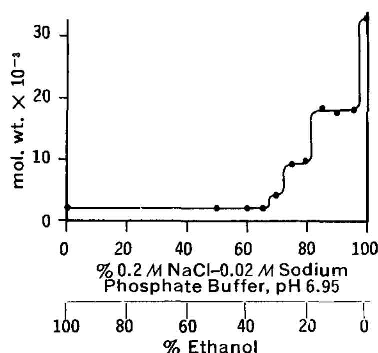


Figure 2—*Molecular weight of diumycin A (10 mg./ml.) in mixtures of ethanol and 0.2* M *NaCl–0.02* M *sodium phosphate buffer, pH 6.85.*

Molecular Weights—Molecular weights were determined by the Archibald approach-to-sedimentation equilibrium method (10), using 5 to 10 mg. solute/ml. solvent. The concentration gradients at the meniscus and cell bottom were measured from schlieren patterns recorded on Kodak metallographic plates and enlarged 10-fold on a Nikon magnifier. Total concentration was measured with the aid of a capillary centerpiece in an interference cell. The integral of the concentration gradient was evaluated by the procedure of Engelberg (11). The partial specific volume of 0.61 cm.³/g., calculated for diumycin from density data using methodology described by Schachman (12), was decreased by 0.01 cm.³/g. for calculations of molecular weight when buffered 8 *M* urea, 9 *M* formamide, or 5 *M* guanidinium chloride was used as the solvent (13).

RESULTS AND DISCUSSION

Although elemental analysis of diumycin, including the determination of phosphorus, indicates a molecular weight of approximately 1900 daltons (1), the apparent molecular weight of diumycin in neutral pH buffer, at a concentration of 3 to 10 mg./ml., is 32,000 daltons (Table I). A minor component has an apparent molecular weight of 65,000 daltons. Dilution disrupts the aggregate (Fig. 1), with several species seen at concentrations below 3 mg./ml. (Table I).

Aggregation in aqueous solvents is observed between pH 2.2 and 12.4, as well as in 1% sodium dodecyl sulfate (Table I). The ineffectiveness of sodium dodecyl sulfate as a disaggregating agent indicates little or no increase in net negative charge on diumycin due to binding of anions (14), with repulsive forces essentially unchanged. The methyl ester and acetate (10 *M* acetate/ 1 *M* diumycin) of diumycin aggregate in aqueous buffer with molecular weights of 32,000 and 38,000 daltons, respectively (Table I). The stability of the 32,000-dalton aggregate under these conditions indicates that ionic forces (noncovalent interactions between polar groups), including salt linkages (14, 15) and hydrogen bonds (16, 17), contribute only slightly to stabilize the 32,000-dalton aggregate. Some involvement of ionic forces is indicated by the molecular weight of 17,000 daltons in 5 *M* NaCl–0.02 *M* phosphate buffer (pH 6.85) and the higher concentration of the 65,000-dalton aggregate than the 32,000-dalton aggregate at pH 2.2 and 4.05 (Table I), a reversal of the relative concentrations of the aggregates that are found in solutions containing 0.2 *M* NaCl.

Stabilization of the aggregate by hydrophobic bonding (18–20) and, in particular, by lipid–lipid hydrophobic bonding (21) is indicated by: (*a*) the ability of the hydrophobic bond-breaking

Table I—Molecular Weights[a] of Diumycin and its Derivatives in Various Solvents

Compound	Concentration, mg./ml.	Solvent	Molecular Weight, daltons Major Component	Minor Component
A, Mixture[b]	10–3	0.2 *M* NaCl–0.02 *M* Na phosphate buffer, pH 6.85 (P[c])	32,000	65,000
A, Mixture	2–0.5	P[c]	32,000	7,000
A, Mixture	0.25	P[c]	1,600	
B	5	90% ethanol–10% P[c]	1,600	
B	5	P[c]	61,000	30,000
Mixture	5	pH 2.2, glycine–HCl buffer, 0.05 *M*	62,000	32,500
Mixture	5	pH 4.05, acetate buffer, 0.1 *M*	65,000	27,000
Mixture	5	pH 10.4, glycine–NaOH buffer, 0.05 *M*	30,200	
Mixture	5	pH 12.4, glycine–NaOH buffer, 0.2 *M*	31,000	
Mixture	5	Isotonic buffer, containing K^+ Na^+, Ca^{2+}, Mg^{2+}, Cl^-, $SO_4^=$, and $PO_4^≡$	30,800	
Mixture	10	Sodium dodecyl sulfate in P[c]	64,000	
Methyl ester of diumycin A	5	90% ethanol–10% P[c]	1,800	
		P[c]	31,000	
Acetate of diumycin A	5	P[c]	38,000	
Mixture	5	5 *M* NaCl in 0.02 *M* phosphate buffer (pH 6.85)	17,000	
Mixture	5	95% P–5% methanol	34,000	67,000
A, Mixture	5	95% P–5% ethanol	18,000	
Mixture	5	95% P–5% *n*-propanol	9,000	14,000
Mixture	5	95% P–5% *n*-butanol	3,800	
A, Mixture	5	90% ethanol–10% P[c]	1,800	
Mixture	5	5 *M* guanidinium chloride	3,600	1,900
Mixture	5	9 *M* formamide	1,600	
Mixture	5	8 *M* urea	1,900	
Partial hydrolysate of diumycin A (lipidless)	5	90% ethanol–10% P[c]	1,200	400
		P[c]	1,400	

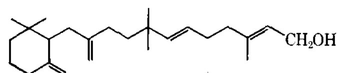
[a] Precision is within 5% accuracy with 15% in low ionic strength solvents. [b] Mixture = diumycin A and diumycin B. [c] P is 0.2 *M* NaCl–0.02 *M* sodium phosphate buffer, pH 6.85.

solvent systems of buffered 8 *M* urea, 9 *M* formamide, or 5 *M* guanidinium chloride to disrupt the aggregate (Table I); (*b*) the inability of a lipidless diumycin to aggregate (Table I); and (*c*) the increasing efficacy of alcohols as disaggregating agents as the length of their hydrocarbon portion increases (Table I). *n*-Butanol is the most effective and methanol is the least effective disaggregating agent, as measured by the apparent molecular weight of diumycin in 95% NaCl–phosphate buffer–5% alcohol (ROH), where R = $—CH_3$, $—CH_2CH_3$, $—CH_2CH_2CH_3$, or $—CH_2CH_2CH_2CH_3$.

The molecular weight of diumycin A (10 mg./ml.) in various mixtures of ethanol and 0.2 *M* NaCl–0.02 *M* sodium phosphate buffer (pH 6.85) is illustrated in Fig. 2. As the concentration of ethanol increases, the molecular weight decreases from 32,000 daltons to 17,000, 10,000, 4000, and finally 2000 daltons. This indicates that: (*a*) disaggregation proceeds by repeated halving of the molecule; (*b*) 16 subunits comprise the 32,000-dalton aggregate; and (*c*) the monomeric concentration term in an equilibrium expression would have an exponent of 16 and 32 for the 32,000-dalton and 65,000-dalton aggregates, respectively, if it is assumed that the law of mass action is valid in these solvent systems.

Table II—Products of Acid Hydrolysis of Diumycin

Glucose
Glucosamine (2 equivalents)
2-Amino-1,3-cyclopentanedione[a]
Acetic acid (2 equivalents)
NH_3 (3 equivalents)
Unknown sugar
H_3PO_4



[3,8,8-trimethyl-11-methylen-12-(2,2-dimethyl-6-methylenecyclohexyl)-2,6-dodecadien-1-ol (diumycinol)]

[a] From diumycin A.

Table II lists the products of the acid hydrolysis of diumycin which includes a 25-carbon lipid (3,8,8-trimethyl-11-methylen-12-(2,2-dimethyl-6-methylenecyclohexyl)-2,6-dodecadien-1-ol) and three hydrophilic sugars. The 32,000-dalton aggregate may be depicted as a core of 16 interacting lipid side chains surrounded by the hydrophilic moieties. This picture is in accord with the X-ray diffraction studies of Luzzati (22), who found that such complex lipids as lysolecithin and phosphatidylethanolamine aggregated in aqueous solution. The aggregate became more spherical as the size of the hydrophilic portion increased.

The 65,000-dalton aggregate may result from the ionic bonding (electrostatic interaction) of two 32,000-dalton aggregates. A less likely interpretation is that a second spherical conformation exists which can accommodate exactly twice the number of lipid side chains found in the 32,000-dalton aggregate.

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Utilization of a Model Copolymer to Evaluate the Contribution of Hydrophobic Bonding in Drug Binding

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Abstract ☐ A copolymer, having an overall composition of 1 unit of vinylpyridine to 26 units of vinylpyrrolidone, was synthesized. Several alkyl copolymers were made by quaternizing the nitrogen of vinylpyridine units of the copolymer with normal alkyl bromides, ethyl through hexyl. Using an equilibrium dialysis procedure, the interaction of *p*-toluene sulfonic acid sodium with each alkyl copolymer was studied over a temperature range of 15–45°, and the binding constants and thermodynamic parameters were determined. The quaternized vinylpyridine units of the alkyl copolymers constituted the binding sites for the *p*-toluene sulfonate ions. The stability of the complex formed in the reaction increased with an increase in temperature from 15–37° but decreased at 45°. The binding process was found endothermic and associated with positive entropy effects, indicating that the polymeric model system described here demonstrated properties expected for hydrophobic bonding. The effect of urea on the binding of sulfonate ion by one of the alkyl copolymers was studied and found to be in keeping with the claim that urea breaks hydrophobic bond.

Keyphrases ☐ Vinylpyridine–vinylpyrrolidone copolymers—synthesis ☐ Equilibrium dialysis—copolymer-*p*-toluene sulfonic acid sodium interaction ☐ Binding sites—copolymer-*p*-toluene sulfonate ions ☐ Urea effect—alkyl copolymer binding sulfonate ions ☐ UV spectrophotometry—identity

During the past 3 decades, several interactions have been reported which involved binding of drugs by plasma proteins; notable examples are penicillins (1), sulfonamides (2–5), methyl orange (6–7), and short- and long-chain fatty acids (8–14). In connection with these binding studies, it has been reported that the interactions primarily take place through ionic forces, but a further contribution to the stability of protein–drug complex is made by the hydrophobic part of a drug molecule. It has also been noticed that the larger the hydrophobic group of a drug molecule, the more stable is the complex. The contributions of hydrophobic groups is attributed to van der Waals forces. However, a close examination of the thermodynamic data has revealed that van der Waals interactions alone cannot account for the stabilizing effect of hydrophobic groups. It is felt that hydrophobic bonding probably plays an important role in drug–protein complexing.

Hydrophobic bonding is a concept introduced by Kauzmann (15), who postulated its thermodynamic properties by extrapolating the behavior of small-size hydrocarbons in an aqueous medium. A hydrophobic bond is defined as the tendency of hydrophobic groups, mainly the hydrocarbons, to adhere to one another in an aqueous solution. The adherence of hydrophobic groups in an aqueous medium is not thought to be merely a manifestation of van der Waals forces; but the structure of water in close proximity to hydrophobic groups is believed to play a significant role, since such adherence processes are accompanied by entropy effects. The concept of hydrophobic bonding was originated to indicate its contribution in stabilizing the folded configuration of globular proteins. On the basis of a physical model, Nemethy and Scheraga (16) have shown that a hydrophobic bond can be formed between two isolated side chains attached to a rigid peptide backbone of protein. Attempts have been made to estimate the thermodynamic contribution of hydrophobic groups to form a drug–protein complex, but the simultaneous contribution of protein molecules due to their "configurational adaptability" obscured such evaluation (17, 18).

It is known that in the event of drug–protein interaction, the thermodynamic activity of a drug in the body is reduced, the biological action of a drug is influenced, and even the metabolism and excretion are hindered. The extent of participation of hydrophobic bonding in exerting such effects is not completely understood. However, one may expect that the concept of hydrophobic bonding can be applied to advan-