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Keyphrases

Indoloquinoline derivatives of lysergic acid
Hypotensive activity—lysergic acid derivatives
Mechanism—hypotensive activity
α-Receptor blockade—lysergic acid derivative

Micellar Solubilization of Testosterone II

In Aqueous Solutions of Some Ionic Surfactants

By ARVIND L. THAKKAR* and NATHAN A. HALL

The solubility of testosterone at 25° was determined in aqueous solutions containing varying concentrations of dodecyltrimethylammonium bromide (DTAB), hexadecyltrimethylammonium bromide (HTAB), and potassium laurate (KL). After the initial stages of solubilization, a linear relationship was observed between the amount of testosterone solubilized and the molar concentration of the surfactant. The order of increasing solubilizing capacity was DTAB < HTAB < KL. The environment of solubilized testosterone, as investigated by the *Z*-value method, was found to be quite polar in all cases. At low concentrations of DTAB and HTAB the *Z* value was similar to that in water. As the concentration of the quaternary ammonium bromides increased, a precipitous drop in *Z* value, corresponding to a sudden decrease in environmental polarity, was observed in the region of the critical micelle concentration. With further increase in the concentration of DTAB and HTAB the *Z* value remained reasonably constant. In low KL concentrations the *Z* value of the testosterone environment was higher (more polar) than in water. With increasing concentrations of KL the *Z* value displayed behavior similar to that in DTAB and HTAB. In general, solutions of ionic surfactants showed higher *Z* values than those of the nonionic surfactants examined previously.

THE MICELLAR solubilization of testosterone by solutions of the nonionic surfactants, polysorbates 20, 40, and 60, was the subject of a prior report from this laboratory (1). The solubilizing capacities of the three polysorbates were determined and the polarity of the chromophore of testosterone examined in these systems. A description of the *Z*-value method for empirically measuring the environmental polarity of the solubilized testosterone was included and the results were discussed in the light of current micellar theories.

In 1949 Ekwall and Sjöblom (2) prepared clear aqueous solutions of testosterone in 10% sodium oleate, 20% sodium myristyl sulfate, and 20% sodium cholate solutions, but they did not study the micellar solubilization of this steroid in detail. Recently Lach and Pauli (3) reported the solubilizing action of aqueous sodium desoxycholate solutions upon this steroid. These workers attributed the solubilizing action of sodium desoxycholate, at least in part, to channel-like inclusion complex formation. Besides these two reports no data regarding the solubilizing action of other anionic or cationic agents for testosterone are available. The investigations reported here include the solubilizing capacities of dodecyltrimethylammonium bromide, hexadecyltrimethylammonium bromide, and potassium laurate, as well as the effect of varying concentrations of these surfactants upon the environmental polarity of solubilized testosterone as determined by the *Z*-value method.

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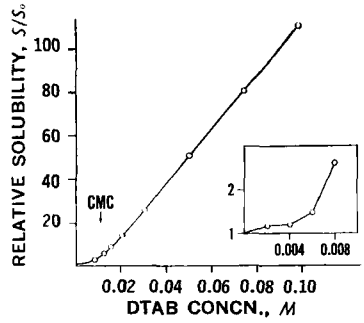

Fig. 1—Relative solubility of testosterone versus *concentration of dodecyltrimethylammonium bromide. (Relative solubility,* S/S_0 = *solubility in surfactant solution,* S/ *solubility in water,* S_0.) *The CMC indicated is the reported (4) value determined in the absence of any solubilizate.*

EXPERIMENTAL

Materials—Dodecyltrimethylammonium bromide (DTAB) and hexadecyltrimethylammonium bromide (HTAB) (K and K Laboratories, Plainview, N. Y.) were recrystallized twice before they were used, DTAB from acetone and HTAB from a chloroform–acetone mixture. The critical micelle concentration (CMC) of DTAB at 25° has been reported as 1.6×10^{-2} mole/l. by Scott and Tartar (4). The CMC of HTAB was calculated from the linear plot of log CMC *versus* carbon atom chain length from the data of these authors as 8.4×10^{-4} mole/l. A 0.1 *M* stock solution of DTAB was readily prepared at room temperature. When preparing a 0.01 *M* stock solution of HTAB, however, it was necessary to apply gentle heat. The dissolved HTAB did not precipitate from solution when cooled to room temperature. From the stock solutions a series of solutions were prepared by appropriate volumetric techniques.

The preparation of solutions of potassium laurate (KL) has been described previously (5). The CMC of KL has been reported as 2.34×10^{-2} mole/l. (6).

Testosterone NF,[1] obtained as micronized crystalline powder, was used in this investigation.

Solubility Determinations—The solubility of testosterone in aqueous solutions of DTAB, HTAB, and KL at 25° was determined by the procedure described earlier (1). As in the case of solubilization of testosterone in solutions of nonionic surfactants, initial supersaturation was found to occur in all the cases. Following this supersaturation, equilibrium solubility was attained within 48 hr. All the solutions, however, were equilibrated for 96 hr. The seemingly anomalous phenomenon of initial supersaturation in solubilized systems containing testosterone has been investigated in detail and will be the subject of a future paper (7).

Z-Value Determinations—This method for determining the environmental polarity of solubilized molecules has been described previously (1) and was utilized in this study. In most micellar solutions of DTAB, HTAB, and KL, however, the quantity of testosterone solubilized was so large that it was not possible to carry out spectral measurements using undiluted solutions. The effect of solubilizate concentration upon the wavelength of maximum absorbance ($\lambda_{max.}$) and *Z* value has been investigated before, for testosterone in solutions of a nonionic surfactant (1), and for camphor in solutions of both ionic and nonionic surfactants (8, 9). No significant differences were found in the values of these parameters. In view of these studies and the fact that these parameters reflect the solvent polarity, it seemed reasonable to assume that dilution with the appropriate surfactant solution would not alter the values of $\lambda_{max.}$ and *Z*. Dilutions were kept to a minimum and, even with the largest dilution, the amount of testosterone in solution was well above its aqueous solubility. This would ensure that the testosterone molecules would be associated with surfactant micelles. In all the cases where dilutions were necessitated, cells of shorter (1 mm.) path length were used.

[1] Generously supplied by Schering Corp., Bloomfield, N. J.

RESULTS AND DISCUSSION

Solubility Determinations—Figures 1, 2, and 3 show the relationship between relative solubility of testosterone and concentration of DTAB, HTAB, and KL, respectively. As in the case of polysorbates,

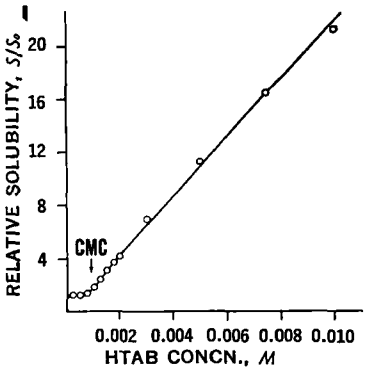

Fig. 2—Relative solubility of testosterone versus *concentration of hexadecyltrimethylammonium bromide. (Relative solubility,* S/S_0 = *solubility in surfactant solution,* S/ *solubility in water,* S_0.) *The CMC indicated is the reported (4) value determined in the absence of any solubilizate.*

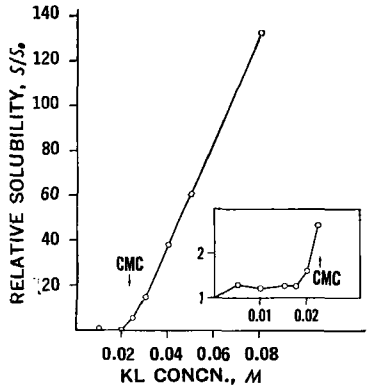

Fig. 3—Relative solubility of testosterone versus *concentration of potassium laurate. (Relative solubility,* S/S_0 = *solubility in surfactant solution,* S/*solubility in water,* S_0.) *The CMC indicated is the reported (6) value determined in the absence of any solubilizate.*

Table I—Solubilizing Capacity of Various Surfactants for Testosterone at 25°

Surfactant	Solubility, Mole Testosterone/Mole Surfactant
Dodecyltrimethylammonium bromide	9.01×10^{-2}
Hexadecyltrimethylammonium bromide	16.26×10^{-2}
Potassium laurate	17.75×10^{-2}
Polysorbate 20	3.529×10^{-2}[a]
Polysorbate 40	3.984×10^{-2}[a]
Polysorbate 60	4.385×10^{-2}[a]

[a] From *Ref. 1.*

the plots are linear after the initial stages of solubilization. The solubilizing capacities were calculated from the slopes of the linear plots and are listed in Table I. In order to facilitate comparison, the previously reported (1) solubilizing capacities of polysorbates 20, 40, and 60 are also listed in Table I.

It may be seen from Table I that among the ionic surfactants the order of increasing solubilizing capacity is DTAB < HTAB < KL. Where surfactant molecules have lipophilic portions of comparable length, the ionic agents are more efficient solubilizers mole for mole than polysorbates, *i.e.*, DTAB and KL are better than polysorbate 20 and HTAB better than polysorbate 60. The anionic KL has the greatest solubilizing capacity of all. Not only is KL more efficient than DTAB and polysorbate 20, but also it is a better solubilizer than HTAB, which is a molecule with a longer lipophilic moiety.

An explanation of the greater efficiency of the fatty acid soap, KL, than that of the quaternary ammonium bromides may lie in the fundamental difference between micelles of the two ionic types. The ions of anionic micelles bear their charges on the exposed oxygen atoms of their polar head groups, the carboxylate groups. For a molecule such as testosterone which has polar–nonpolar characteristics, it is conceivable that hydrogen bonding would be one of the factors in solubilization. The hydroxyl group at C-17 in testosterone would be the site for such bonding. The electron-rich oxygen atoms in the carboxylate ions of KL micelles should be good acceptors for hydrogen. The quaternized nitrogen atom in the polar heads of DTAB and HTAB since it has no unshared electrons would be incapable of hydrogen bonding. The trimethylammonium polar groups will, however, be capable of association with testosterone by ion–dipole and van der Waals type of interactions.

It is interesting to take a closer look at the solubilizing capacities of KL, DTAB, and polysorbate 20. These three surfactants have identical lipophilic moieties within their molecules. In an idealized picture of spherical micelles these lipophilic portions may be considered as forming the relatively nonpolar micellar core which should be similar for micelles of KL, DTAB, and polysorbate 20. If the mechanism of solubilization involved association of testosterone with the nonpolar micellar core, then similar solubilizing capacities would be expected for these three surfactants. However, the solubilizing capacities indicate that KL is about twice as efficient as DTAB and about five times as efficient as polysorbate 20. A similar situation exists for HTAB and polysorbate 60 which have identical lipophilic moieties; HTAB is more than three times as efficient as polysorbate 60. From these differences, it seems logical to believe that solubilization of testosterone may not involve penetration into the micellar interior.

The results of this study and of an earlier one by Bjaastad, Hall, and Thakkar (5) point out that for molecules such as testosterone and camphor which possess a partially polar nature, anionic surfactants are more efficient solubilizers than cationic surfactants. This observation is in apparent disagreement with the generalization that cationic surfactants are better solubilizers than anionic surfactants (6, 10). It is important to take into consideration the nature of the solubilizate and the mode of solubilization before making a generalization regarding the relative solubilizing capacities of different surfactant types. For a nonpolar solubilizate which is solubilized in the lipophilic interior of micelles, for example, cationic agents would be more efficient than anionic agents because the micelles of cationic agents have a greater degree of disorder due to the nature of their ionic head groups (11).

If the concentration of surfactant at which the relative solubility of testosterone begins to increase is considered to be the CMC, then it will be seen from Figs. 1, 2, and 3 (where the CMC's shown are reported values, determined in the absence of any solubilizate) that these values are below the literature values. One might postulate that this early increase in solubility probably results from the formation of mixed micelles between testosterone and surfactant molecules at surfactant concentrations below their usual CMC's and a consequent lowering of the CMC in the ternary system. That such an association between testosterone and the surfactants can occur seems plausible in view of the fact that testosterone also possesses surface activity (12, 13). Wurster and Taylor (14) have shown that the steroid prednisolone is also solubilized in solutions of sodium lauryl sulfate below the binary water-surfactant CMC.

Z-Value Determinations—The relationships among the concentration of DTAB, solubility of testosterone, and *Z* value are shown in Fig. 4. Similar plots for HTAB and KL are shown in Figs. 5 and 6.

In case of the cationic surfactants at low concentrations (up to 0.006 *M* for DTAB, 0.008 *M* for HTAB) in which the solubility of testosterone is not

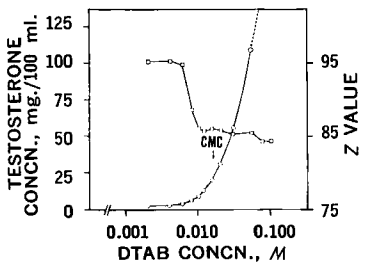

Fig. 4—The relationship between concentration of dodecyltrimethylammonium bromide (logarithm), solubility of testosterone (○), and Z value (□). The CMC indicated is the reported (4) value determined in the absence of any solubilizate.

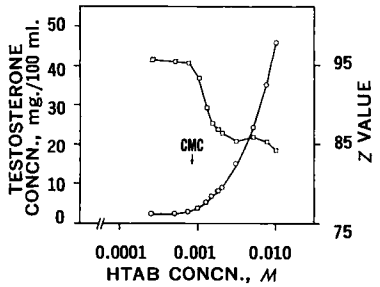

Fig. 5—The relationship between concentration of hexadecyltrimethylammonium bromide (logarithm), solubility of testosterone (○), and Z value (□). The CMC indicated is the reported (4) value determined in the absence of any solubilizate.

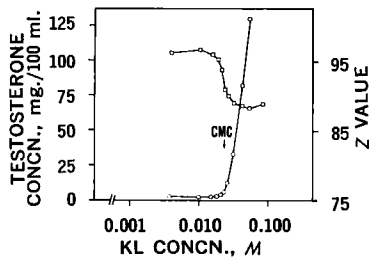

Fig. 6—The relationship between concentration of potassium laurate (logarithm), solubility of testosterone (○), and Z value (□). The CMC indicated is the reported (6) value determined in the absence of any solubilizate.

appreciably greater than in water, the Z value is similar to that in water. However, as the solubility of testosterone begins to increase, the Z value drops sharply over a narrow range of surfactant concentration. After this sudden drop the Z value appears to attain a plateau. It is interesting to note that the relatively precipitous drop in Z value takes place as the solubility of testosterone begins to increase. The solubility of testosterone increases steadily with increasing surfactant concentration in both DTAB and HTAB solutions. Unlike the situation with the polysorbates (1), the increase in the solubility of testosterone does not parallel the change in Z value.

The anionic surfactant, KL, displays a Z-value behavior similar to the cationic surfactants. A sudden drop in Z value takes place in the region of the reported CMC just as the solubility of testosterone begins to increase. The Z value in low concentrations of KL (0.005 M and 0.01 M) is slightly higher than that in water.

As pointed out earlier (1, 8), the decrease in Z values with increase in surfactant concentration may reflect the increase in concentration and organization of the micelles. That this decrease in Z value takes place at or near the CMC is significant. The rather abrupt formation of micelles would indeed lead to an increased structural organization within the solution.

The relative regions of solubilization in or on the micelle may be deduced by a comparison of the changing Z values in the ionic surfactant solutions and in the nonionic polysorbate solutions (1). Higher Z values are attained with all the three ionic surfactant solutions than with polysorbates. The concentrations of ionic surfactants employed in this investigation are much higher on a molar basis than those of the polysorbates used in the previous investigation. The quantity of testosterone solubilized by ionic surfactants is also greater than that solubilized by the polysorbates and yet the solubilized testosterone is in an environment of higher polarity.

The Z value, when it becomes reasonably constant in more concentrated ionic surfactant solutions, compares with the Z value in methanol–water or ethanol–water mixtures. This indicates that the environment of the carbonyl chromophore of the solubilized testosterone is more polar than methanol or ethanol. In view of this high polarity, it seems logical to believe that the carbonyl chromophore is associated with the polar head groups of the surfactant. In the case of the anionic, KL, for which hydrogen bonding between the ionized carboxylate group and the hydroxyl group at the C-17 of testosterone may be postulated, this would mean that the carbonyl chromophore at C-3 is also associated with this polar region of the micelle. If idealized spherical micelles are considered, then this region would be the micellar surface. Such a simplified picture is not easy to visualize for the cationic agents DTAB and HTAB where hydrogen bonding of the hydroxyl group of testosterone cannot take place with the quaternary ammonium polar head groups. From the available Z-value data it can only be concluded that the carbonyl chromophore is in an environment more polar than methanol–water or ethanol–water mixtures. It is not impossible in case of the cationic surfactants that the nonpolar portion of the testosterone molecule could be associated to some extent with the relatively nonpolar palisade region of the micelles. However, as the high Z values indicate the carbonyl chromophore must remain in the polar environment around the polar head groups.

The observation that solutions of KL provide an environment of higher polarity than do the solutions of DTAB and HTAB is in agreement with the findings of Bjaastad and Hall (8) in the case of solubilized camphor and 2-heptanone. An explanation of this observation could lie in the nature of the polar head groups of KL on the one hand and of DTAB and HTAB on the other.

The surface of the KL micelles would be rich in carboxylate ions, and since these ions arise from a weak acid, some hydrolysis would be expected in an aqueous medium. The resulting removal of protons should contribute to the polarization of the environment of the solubilizate (elevated Z value). Hydrogen bonding, possible with KL but not with DTAB or HTAB, may also alter the polarity of the medium at the polar head groups. Furthermore, the carboxylate ions have their charges exposed for direct interaction with the solubilizate chromophore, while the ionic charge on the nitrogen atom of the quaternary ammonium ions is shielded from close contact with the solubilizate by the presence of methyl group substituents. Thus, it appears that these several factors could result in KL solutions producing a more polar environment for the solubilizates than the quaternary ammonium surfactants.

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Keyphrases

Testosterone micellar solubilization
Ionic surfactants—testosterone solubilization
Z values—determination
Surfactant concentration effect—Z values

Interaction of NO_2 with Monolayers of Phospholipids Extracted from *E. coli* at 15 and 37°

By N. D. WEINER, M. AMANAT, D. BLONDO, R. CAPRIOLI, N. DINERMAN, and A. FELMEISTER*

Phospholipids were extracted from *E. coli* grown at 15 and 37°. The fatty acid residues of the 15° phospholipids were found to be considerably more unsaturated than the 37° phospholipids. These phospholipids were spread as monomolecular films and exposed to NO_2-containing atmospheres. Whereas the 37° phospholipid films showed no interaction, NO_2 was found to expand considerably the 15° phospholipid films. The results demonstrate that simple changes in environmental conditions may affect markedly the interaction of air pollutants such as NO_2 with biological membranes.

ISOLATION and characterization of membrane components from a wide variety of organisms indicate that the compositions of the membrane phospholipids differ significantly, not only from organism to organism, but even from tissue to tissue within a single organism (1). The phospholipid composition of the membranes of a number of microorganisms has also been shown to vary considerably with varying growth conditions. For example, Engleman, Terry, and Morowitz (2) point out that the fatty acid residues of the membrane phospholipids of *Mycoplasma laidlawii* are a function of the fatty acids included in the growth medium. Marr and Ingraham (3) demonstrated that the degree of unsaturation of membrane fatty acids of *E. coli* is dependent on growth temperature. These latter workers pointed out that the increase in unsaturation of the fatty acids of *E. coli* grown at lower temperatures is part of an expected adaptive process. The degree of fluidity exhibited at low temperatures by these unsaturated acids compares to that of their saturated counterparts at normal growth temperatures. Thus, the organism is able to maintain the level of membrane transport and other essential processes even at temperatures well below the normal optimum level.

In previous studies of the interaction of air pollutants with monomolecular films, it was noted that NO_2 expanded monolayers of egg lecithin (where 50% of the fatty acid groups contain at least one unsaturated bond), but did not affect monolayers of synthetic dipalmitoyl lecithin. The expansion apparently is the result of a chemical interaction of NO_2 with the double bonds of the unsaturated fatty acid groups of egg lecithin rather than a simple physical penetration into the film (4).

In view of these latter results and Marr and Ingraham's report, it was of interest to determine whether the membrane phospholipids extracted from *E. coli* grown at 15 and 37°, respectively, would exhibit similar differences in their interaction with an air pollutant such as NO_2.

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