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# STUDIES ON SULFAETHIDOLE - LYSOZYME INTERACTIONS BY FLUORESCENCE QUENCHING

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## ABSTRACT

Fluorescence quenching studies on sulfaethidole - lysozyme interactions, demonstrate that, the binding parameters for the drug are greatly influenced by alteration in protein concentration, as well as, changes in pH of the medium. On increasing lysozyme concentration, the stoichiometry of the reaction changes asymptotically, whereas, no significant change does occur in the other binding parameters. Furthermore, the binding affinity of the drug shows a pronounced increase, as the pH is reduced from 8 to 5.0, while the stoichiometry of the reaction decreases, as the pH is shifted towards the acid region.

Analysis of the data obtained in conjunction with alteration of hydrogen-ion concentration, provide evidences that, the negative charge on sulfaethidole molecules is not an essential factor for



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the drug - lysozyme interactions. In addition, it become ascertained that, the hydrophobic force rather than the electrostatic one, is the most form of energy responsible for the binding of the drug to lysozyme.

## INTRODUCTION

The binding of ophthalmic drugs to tear proteins, has been recognized as an important factor in drug availability, drug efficacy and drug transport (1).

The tear proteins consist mainly of six fractions namely. albumins (30%), globulins (40%) and lysozyme (30%) (2). To the latter fraction, the antibacterial properties of the tears is referred.

Although considerable information has been amassed on drug - albumin interactions (3,4), however, there is scarcity of materials on the binding of drugs to lysozyme, especially ophthalmic ones (5).

In a preceding communication, the utility of the fluorescence quenching as a reliable technique for detection of drug - lysozyme interactions and quantitative determination of the different binding parameters, has been well established (6). Furthermore, using the-same technique, the binding of a wide range of ophthalmic drugs to lysozyme was also recently investigated by El-Nimr et al (7,8).

The objective of the present work is twofold. To investigate the binding affinities of the acid drug sulfaethidole with a pKa 5.6 (9), to lysozyme in relation to certain criteria of major significance from both pharmaceutical, as well as, pathological points of view.

These factors include, effect of some formulation requirements i.e. variation in hydrogen-ion concentration of ophthalmic solutions and alteration in lysozyme titre which may arises due to disease states (10). Secondly, to provide additional evidence on the mechanism of sulfonamides - lysozyme interactions, which may introduce a further support for our previous assumption on sulfamethazine - lysozyme interaction model (8).

## EXPERIMENTAL

### Materials and Methods

Hen egg-white lysozyme, 2 times crystallized, lot No. E2-3431 (specific activity 14,170 units/mg), Schwarz/Mann, Orangeburg, N.Y. Sulfaethidole was a gift of Smith Klyne and French Laboratories , Philadelphia, PA. All other materials were reagent grade. Deionized water was used throughout.

Lysozyme and drug - lysozyme solutions were prepared in 0.1M phosphate buffer. The molecular weight of the enzyme was taken at 14,400 (11,12). The different pH's were adjusted with a Beckman Digital pH-meter, model 4500 (Beckman, Fullerton, CA). Other experimental conditions were adopted as previously reported (6).

The fluorescence measurements were carried out at 22$^{o}$C, on a Perkin Elmer MPF-44A spectrofluorimeter (Perkin-Elmer, Norwalk, CT); using square quartz cells 1-cm path length. The excitation wavelength was 305 nm (13), with a slit width of 6 nm, and the emission was scanned using a slit width of 7 nm. For all lysozyme and

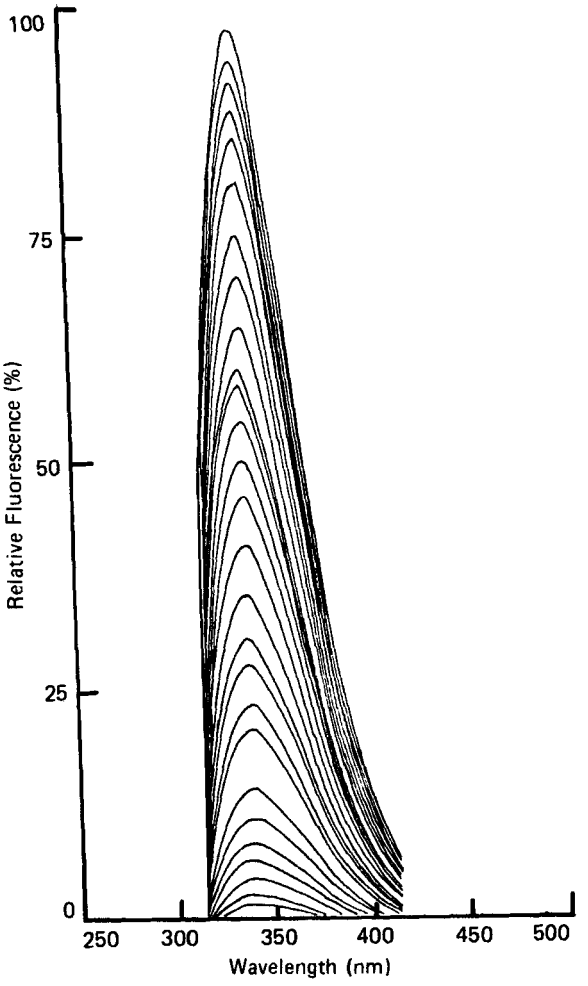


FIGURE 1

Fluorescence spectra of lysozyme and sulfaethidole - lysozyme complexes excited at 305 nm and 22°C. Lysozyme concentration 1 X $10^{-4}$M, in 0.1M phosphate buffer and pH 6.2

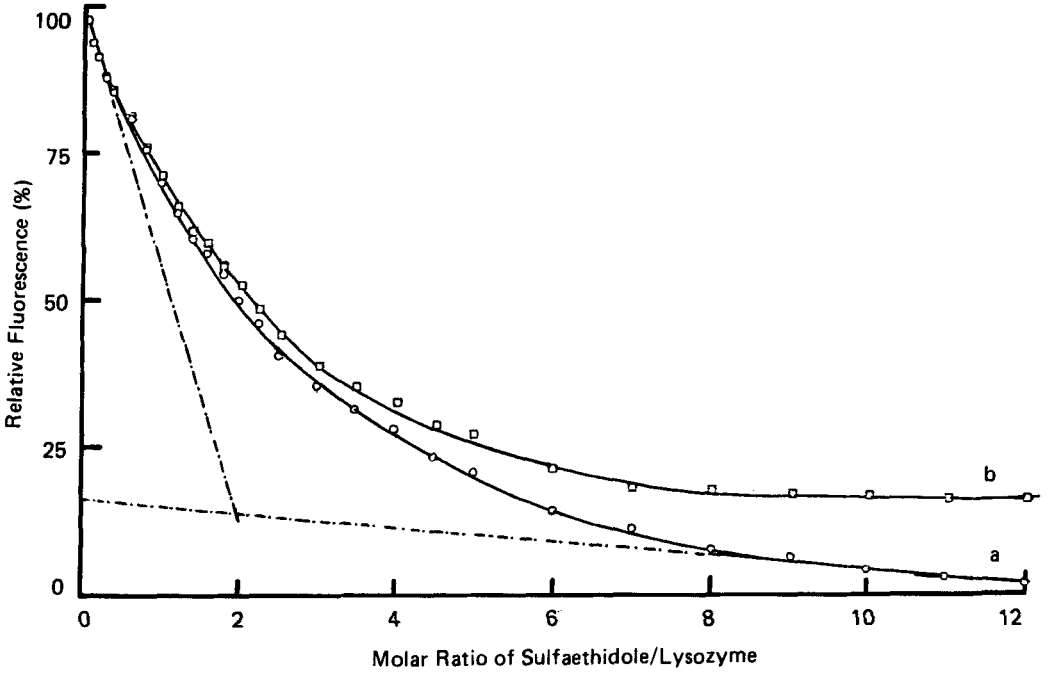


FIGURE 2

Relative fluorescent emission at 337 nm and 22°C, as a function of the mole ratio of sulfaethidole to lysozyme. Lysozyme concentration 1 X $10^{-4}$M, in 0.1M phosphate buffer and pH 6.2 The intersection of the terminal slopes is at a mole ratio = 2

○ - Experimental curve (a) and □ - Corrected curve (b)

drug - lysozyme solutions, the emission peak was found to be near 337 nm. Figure 1 indicates that, in no instance did the drug emit at any investigated wavelength or cause any significant shift in the emission maxima.

The stoichiometry of the reaction (n) was determined graphically by plotting the relative fluorescence intensity against drug to protein mole ratio (Fig. 2). The other binding parameters

namely, the binding constants (K) and the free heat of formations ( $\Delta$ G), were then calculated (6), after carrying out the necessary corrections (14,15), on experimental data (Fig. 2-a),to give the - corrected curve (Fig. 2-b) .

## RESULTS AND DISCUSSION

The effect of protein concentration on sulfaethidole - lysozyme interactions at pH 6.2 is shown in Table 1. It is apparent that. at low lysozyme concentrations, the stoichiometry of the reaction(n) shows a great increase, whereas the mole ratio at the stoichiometric point was found to decrease gradually at relatively higher protein concentrations. This finding is in consistence with the observation of Attallah and Lata (15), on steroid - albumin interaction model. As regards the other binding parameters viz. the binding constants(K) and the free heat of formations ( $\Delta$ G), the data obtained show in all cases, no significant changes, suggesting thus the independency of

TABLE 1

Binding Parameters for Sulfaethidole - Lysozyme Interactions, as a Function of Protein Concentration , at 22$^{O}$C and pH 6.2

| Lysozyme concn. (M) | Sulfaethidole/lysozyme mole ratio (n) | K ($M^{-1}$) | $\Delta$ G (Kcal $M^{-1}$) |
|---|---|---|---|
| $5.0 \times 10^{-5}$ | 2.0 | 12,900 | -5.55 |
| $1.0 \times 10^{-4}$ | 2.0 | 13,000 | -5.55 |
| $1.5 \times 10^{-4}$ | 1.0 | 12,900 | -5.55 |
| $3.472 \times 10^{-4}$ | 0.5 | 13,600 | -5.58 |

such parameters on protein concentration over the investigated concentration range ( $5 \times 10^{-5}$ - $3.472 \times 10^{-4}$M).

Table 2 and Figure 3, demonstrate also the effect of hydrogen-ion concentration, on the binding affinity of sulfaethidole to lysozyme at a fixed protein concentration ($3.472 \times 10^{-4}$M)

It would appear from Table 2 that, any change in the hydrogen - ion concentration of the medium, is deeply reflected on the different binding parameters. The stoichiometry of the reaction was found to decrease at lower pH-values, whereas, increases as the pH is shifted towards the alkaline region. Graphic representation of the binding constants, as a function of pH, on a semi-log scale reveals that, the binding constant of the drug increases linearly as the pH is reduced from 8 to 5.0 or as the drug becomes unionized (Fig. 3).

In the light of the findings obtained, it is evident that, for an acid drug like sulfaethidole with a pKa 5.6 (9), the negative

TABLE 2

Binding Parameters for Sulfaethidole - Lysozyme Interactions, as a Function of pH at 22°C. Lysozyme Concentration $3.472 \times 10^{-4}$M, in 0.1M Phosphate Buffer.

| pH | Sulfaethidole/lysozyme mole ratio (n) | K ($M^{-1}$) | $\Delta$ G (Kcal $M^{-1}$) |
|---|---|---|---|
| 5.0 | 0.5 | 24,500 | -5.93 |
| 6.2 | 0.5 | 13,600 | -5.58 |
| 7.4 | 1.0 | 8,500 | -5.30 |
| 8.0 | 1.0 | 6,300 | -5.13 |

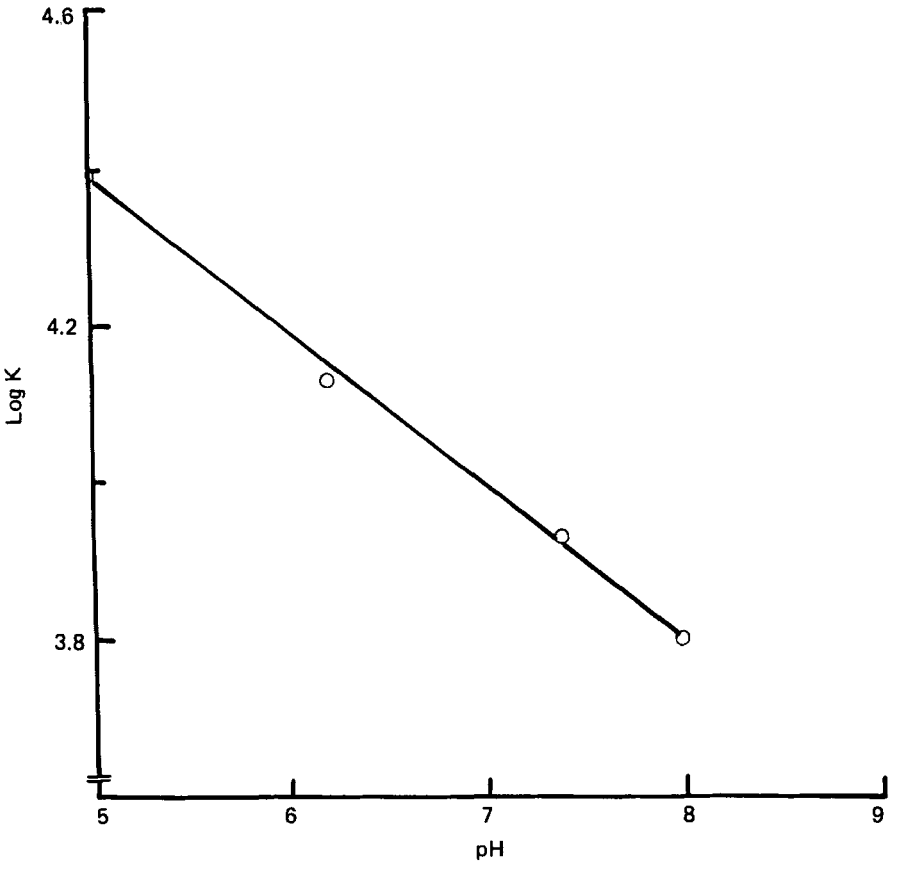


FIGURE 3

Plot of log binding constants for sulfaethidole - lysozyme interactions, as a function of pH at 22°C. Lysozyme concentration $3.472 \times 10^{-4}$M, in 0.1M phosphate buffer.

charge is not an essential factor for the binding of the drug to the cationic lysozyme molecules. In contrast, the unionized species were found to have a binding constant to lysozyme of at least an order of magnitude more than the anions.

The data obtained in conjunction with the pH-rate profile, appears also to put to rest enough evidence on the mechanism of sulfonamides -

lysozyme interactions, which support the previous assumption proposed recently by El-Nimr et al (8), on sulfamethazine - lysozyme interaction model. The authors stated that, for such type of interactions, the-binding forces are not primarily due to electrostatic interactions, as formerly pointed out (16,17), but mainly attributed to hydrophobic phenomena. These findings receiving again an increasing support from another view point which indicate that, the hydrophobic force is the most form of energy responsible for the binding of sulfonamides to proteins (18 - 20).

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