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Effect of surfactant monomers on chloramphenicol association to an albumin-lecithin complex: a model for modified drug absorption

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The binding of chloramphenicol to an albumin-lecithin complex in the presence or absence of premicellar concentrations of both ionic and non-ionic surfactants has been examined. Long chain, strong ionic detergents, such as sodium dodecyl sulphate or cetyltrimethylammonium bromide, severely perturb protein structure and eventually allow full separation of the complex into lecithin and albumin-detergent complexes. The dissociation process is reversible upon the removal of the detergent by exhaustive dialysis. After the splitting of the complex, the amount of antibiotic associated with the lipid-protein mixture increases. Structural alteration of the albumin-lecithin complex and the increase in the binding of chloramphenicol have an effect on the transfer rate of this antibiotic across an artificial barrier consisting of an aqueous dispersion of the same complex, as observed in a model system. It is suggested that a reversible alteration in membrane structure, and consequently in membrane permeability, might be easily effected, at the molecular level, through a reversible dissociation of structural lipoproteins into their components, operated by premicellar concentrations of ionic surfactants. This represents a tentative picture of the possible events taking place within the membrane and modifying the absorption rate of a drug, when it is associated with surfactants in a pharmaceutical preparation.

The possible influence of premicellar concentrations of surfactants on drug absorption and particularly those transport mechanisms which presumably involve structural changes in the biological membrane has attracted attention (Gibaldi & Feldman, 1970; Gibaldi & Grundhofer, 1972a,b). The extensive literature (Steinhardt & Reynolds, 1969) pertaining to the reaction of protein with ionic detergents and the subsequent effect on protein conformation, suggested to us that surfactants might influence drug absorption by affecting the molecular arrangement and stability of biomembranes.

Previously Alhaique, Marchetti & others (1975) indicated that an allosteric transition (Monod, Wyman & Changeux, 1965; Blumenthal, Changeux & Lefever, 1970) could be easily effected by reaction of surfactant monomers (sodium dodecyl sulphate) with a protein (bovine serum albumin) in the presence of a ligand (chloramphenicol). After the interaction of the anionic surfactant with the protein, a negative cooperative (Wallach, 1972) binding process took place which eventually resulted in an increase of free antibiotic. In diffusion experiments performed in a model system described elsewhere (Alhaique, Marchetti & others, 1972), the negative cooperative process was found to be paralleled by an increase in the transfer rate of the antibiotic.

Nissim (1960a) has pointed out that both sodium dodecyl sulphate and cetyltrimethylammonium bromide have definite structural effects on the gastrointestinal mucosa. The activity of the cationic surfactant is mainly in the cationic part of the molecule (Nissim, 1960a) and in several cases resulted in the inhibition of absorption (Nissim, 1960b).

Bradford, Swanson & Gammack (1964) observed significant increases in recovery of phospholipids or cholesterol in the supernatant liquid of microsomal suspensions from brain tissue, in the presence of micellar concentrations of detergents.

More recently, Feldman, Reinhard & Willson (1973) showed that a physiological surfactant altered the composition of the everted rat intestinal membrane by producing an efflux of membrane components from the everted rat preparation.

Since biological membranes consist of both lipid and protein, we have sought to determine if premicellar concentrations of surfactants have an effect on the binding of a ligand to a lipoprotein complex and eventually on the molecular stability of the complex itself.

The present paper deals with the binding and permeation kinetics of an antibiotic (chloramphenicol) across an artificial barrier consisting of an aqueous dispersion of a synthetic lipoprotein complex, a 2:1 by weight serum albumin–lecithin complex (Botré, Borghi & others, 1970).

MATERIALS AND METHODS

Materials. Crystallized bovine serum albumin, sodium dodecyl sulphate (specially pure) and cetyltrimethylammonium bromide were BDH. The protein (mol. wt 69 000) was dialysed against water for 5 days at 4° and then lyophilized. Sodium dodecyl sulphate and cetyltrimethylammonium bromide were both purified by repeated recrystallizations. Their critical micelle concentrations in water at 25° were $7{\cdot}9 \times 10^{-3}$ and 9×10^{-4} M respectively by the conductance method, in good agreement with literature values (Mukerjee & Mysels, 1971). In a phosphate buffer (Yang & Foster, 1954) pH 6·8, ionic strength 0·2, their critical micelle concentration was lowered to $6{\cdot}1 \times 10^{-3}$ and $4{\cdot}4 \times 10^{-4}$ M respectively, as determined at 25° by the dye method. Polysorbate 80 (Fluka), puriss grade, had a critical micelle concentration $1{\cdot}4 \times 10^{-5}$ g ml^{-1}. Chloramphenicol was the generous gift of the Carlo Erba Co, Milan, Italy. Synthetic L-α-lecithin (Grade I: chromatographed) was from Sigma Chemical Company. A stable aqueous dispersion of lecithin was obtained through the addition of an ether solution of the phospholipid to distilled water, followed by vacuum evaporation of the organic solvent. All other reagents were of analytical grade.

Dialysis experiments. Aqueous dispersions at pH 6·8, ionic strength 0·2, were obtained with a phosphate buffer (Yang & Foster, 1954). This pH value is well above the isoelectric point of the protein, 4·7 (Waugh, 1954). L-α-Lecithin is isoelectric over a wide range of pH (Bangham, 1968). All experiments were completed within 24 h in order to minimize the hydrolysis which dilute solutions of sodium dodecyl sulphate undergo on standing (Pallansch & Briggs, 1954). The binding of detergents to bovine serum albumin is independant of ionic strength, the effect of which appears to be entirely on the concentration of free surfactant monomers (Reynolds & Tanford, 1970). Nevertheless, the equilibrium surfactant concentration in no case exceeded the critical micelle concentration of the detergent at a given temperature, ionic strength (Emerson & Holtzer, 1967) and pH. 2 ml of an aqueous dispersion of the lipoprotein complex (or

of lecithin alone), was placed in a dialysis sac (18/32 type, The Scientific Instrument Centre Ltd) and equilibrated against 5 ml of a solution containing the reagents at 25° ± 0·2° for 18 h. For each series of dialysis experiments, two runs were set up in parallel, each run being the same except for the presence of the second ligand. In this way an ultraviolet analysis of the solution outside the dialysis sacs allowed us to determine, by direct comparison, the effect of the second ligand on the binding of the first to the lipoprotein complex (or to lecithin). The ultraviolet analysis of free chloramphenicol concentrations at equilibrium in the compartment not containing the protein was carried out at the wavelength of 278 nm by means of a Beckmann DU-2 spectrophotometer using quartz cells having a path length of 1 cm. Surfactants were found not to interfere with the assay at this wavelength.

Electrophoretic studies. Electrophoretic analysis was carried out on cellulose acetate strips (Cellogel Chemetron, Milan Italy) 5·7 × 14 cm in size (Bartlett, 1963). The saturating buffer solution was the same as that employed in both dialysis and diffusion experiments (pH 6·8). The applied potential was 100 V, which corresponded to a current of 12 mA. 0·5 μl of the sample was applied to the strips. After 1 h, the strips were removed and placed for 5 min in amido black dye and then washed with a clearing solution (methanol 475 ml, water 475 ml and glacial acetic acid 50 ml). This way the protein was detected and quantitatively determined by densitometry. For this purpose the strip was placed in a transparent sleeve and run through an Analytrol densitometer.

Diffusion experiments. The cell and procedures used were described previously (Alhaique & others, 1972). In all experiments the lecithin, or the lecithin protein aqueous dispersions working as the barrier to the diffusing species, had the same composition as that in the dialysis measurements. A theoretical treatment of a similar diffusion cell was given by Nogami, Nagai & Sonobe (1970).

RESULTS AND DISCUSSION

Plot a of Fig. 1 shows the moles of chloramphenicol bound to the lipoprotein complex as a function of the initial antibiotic concentration in solution, measured by equilibrium dialysis. The interaction between the antibiotic and the complex shows little or none of the characteristics of the binding of a small molecule to a fixed number of sites on a protein. Instead there is a linear relation between the total concentration of chloramphenicol and the moles of it associated with the complex. Furthermore, the

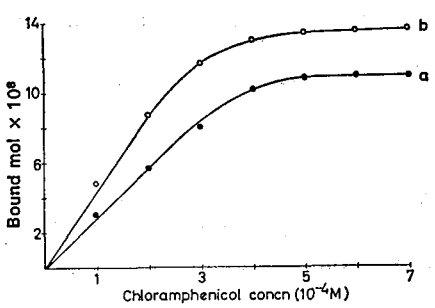

FIG. 1. Association curves of chloramphenicol (CAF) with a 2:1 by weight albumin-lecithin complex (●) and L-α-lecithin (○), in phosphate buffer (pH 6·8) at 25°. Plot a refers to an aqueous dispersion (3% w/v) of the serum albumin-lecithin complex; plot b to an aqueous dispersion (1% w/v) of lecithin.

interaction curve of chloramphenicol with an aqueous dispersion of lecithin in a concentration equal to the relative amount present in the lipoprotein complex (see Fig. 1, plot b) shows a similar trend, suggesting a similar association mechanism. This can be related to a "trapping" effect, like that described for inorganic salts by Bangham (1963) and Bangham, Standish & Watkins (1965), i.e. the antibiotic remains associated with the swollen phospholipid structure and is restricted in its diffusion out of the lipid and thus out of the dialysis sac. It is then reasonable to suppose that the antibiotic is dissolved in a lipophilic portion of the lipoprotein complex rather than being bound to a fixed number of sites.

If premicellar concentrations of sodium dodecyl sulphate or cetyltrimethylammonium bromide are added to an aqueous dispersion of the albumin-lecithin complex in the same experimental conditions, the number of chloramphenicol moles confined inside the dialysis sac at equilibrium increases rapidly (see Fig. 2, plots a and b, and inset, plot a'). This effect might be ascribed to the splitting of the synthetic lipoprotein into lecithin and serum albumin-detergent complexes, each of them capable of binding the antibiotic to a larger extent than the complex itself. The release of lecithin from the bonding sites of the protein follows the well known interaction of ionic surfactants with serum albumin (Pallansch & Briggs, 1954; Few, Ottewill & Parreira, 1955).

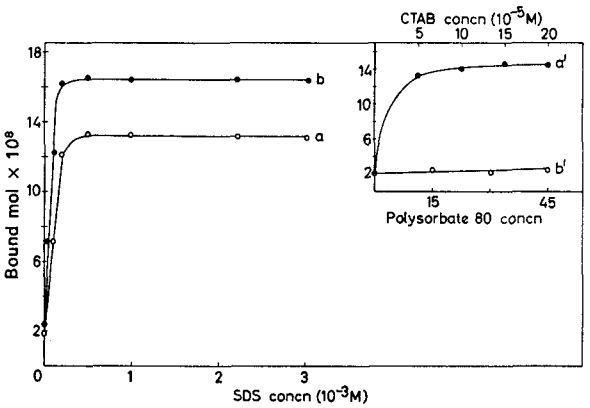

FIG. 2. Association curves of chloramphenicol (ord.) with the albumin-lecithin complex (3% w/v) in phosphate buffer (pH = 6·8) at 25°, in the presence of increasing amounts of sodium dodecyl sulphate (SDS). Plots a (○) and b (●) refer to different initial concentrations of the antibiotic, i.e.—3·1 × 10^{-5} and 6·2 × 10^{-5}M respectively.

Inset. Association curves of chloramphenicol (ord.) with the albumin-lecithin complex (3% w/v) in phosphate buffer (pH 6·8) at 25°, in the presence of increasing amounts of surfactants. Plots a' and b' refer to cetyltrimethylammonium bromide (●) (CTAB) and polysorbate 80 (○) (concn in mg ml^{-1} × 10^{2}) respectively. In all cases, the initial concentration of the antibiotic was 3·1 × 10^{-5}M.

This interpretation of the data is consistent with an electrophoretic analysis which parallels the dialysis measurements. As summarized in Table 1, the fraction of serum albumin bound to lecithin in the absence of surfactants remains constant after a critical protein to lecithin concentration ratio is reached (20:10 by weight). This ratio corresponds to the relative quantities present in the lipoprotein complex (Botré & others, 1970).

When an ionic surfactant, namely sodium dodecyl sulphate, is added to an aqueous dispersion of the complex, the fraction of serum albumin bound to lecithin decreases and eventually reaches zero, as surfactant concentration increases.

Table 1. *An electrophoretic analysis of albumin-lecithin mixtures in different weight ratios in the absence of sodium dodecyl sulphate (SDS) and of the albumin-lecithin complex (2:1 by weight) in the presence of increasing amounts of the same surfactant.*

BSA mg ml^{-1}	Lecithin mg ml^{-1}	SDS mol litre^{-1} $\times 10^5$	BSA bound %
20	—	—	0
20	2	—	17–15
20	5	—	39–33
20	10	—	80–76
20	15	—	81–76
20	20	—	81–76
20	10	5	60–56
20	10	10	24–21
20	10	50	11–7
20	10	100	8–3
20	10	500	6–0

The dissociation of the albumin-lecithin complex is apparently a feature of an *ionic* long chain structure, because a non-ionic compound, i.e., polysorbate 80, gave negative results in the same experimental conditions (see Fig. 2 inset, plot b').

When sodium dodecyl sulphate is added to an aqueous dispersion (3% w/v) of the albumin-lecithin complex to bring about dissociation, and the mixture is allowed to dialyse against buffer until no further surfactant can be removed, an electrophoretic analysis of the mixture indicates that the original complex has been regenerated. It would thus appear that the dissociation of the complex upon the addition of a premicellar concentration of surfactant is reversible. The regenerated albumin-lecithin complex still binds chloramphenicol to a significant extent, i.e. only 5% less than the original complex.

Structural alteration of the albumin-lecithin complex and the subsequent increase in the binding of chloramphenicol have an effect on the transfer rate of the antibiotic across an artificial barrier consisting of an aqueous dispersion of the same complex, as observed in diffusion experiments with a model system described elsewhere (Alhaique & others, 1972).

The permeation lag times and permeability coefficients were estimated from the rate of antibiotic transfer across the barrier under quasi steady state conditions (Lueck, Wurster & others, 1957; Nogami & others, 1970). In Fig. 3 permeability coefficients and permeation lag times are plotted as a function of the lecithin content of the barrier either when this phospholipid is the only component (plots a and a') or when it is mixed with serum albumin in different weight ratios (plots b and b').

After the initial addition of lecithin to the protein solution, the permeability of the barrier to chloramphenicol differs slightly from that of a solution of serum albumin alone. At the same time, the corresponding permeation lag times decrease rapidly indicating that the permeant interacts to a less extent with barrier constituents.

When the protein-lecithin mixture reaches a composition equal to that of the complex, any further addition of phospholipid causes a rapid decrease in permeability and an increase in lag times. In the same experimental conditions and for an aqueous dispersion of lecithin alone, a linear relation exists between the phospholipid concentration and both the rate of antibiotic permeation and lag time (plots a and a' in Fig. 3). This denotes that no singular effect can be ascribed either to concentration or

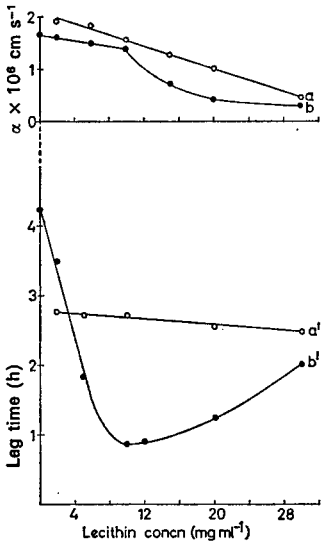

FIG. 3.

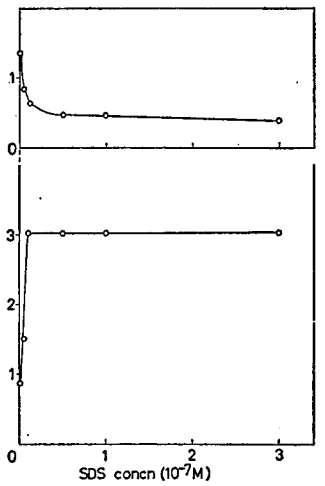

FIG. 4.

FIG. 3. Permeability coefficients (α) and permeation lag times for the antibiotic transfer across the barrier in a diffusion cell, are plotted as a function of the lecithin content of the barrier either when this phospholipid is the only component (plots a and a') (○) or when it is mixed with a constant amount (2% w/v) of bovine serum albumin (plots b and b') (●).

FIG. 4. Permeability coefficients (α) and permeation lag times for the antibiotic transfer across the barrier, are plotted as a function of increasing amounts of sodium dodecyl sulphate (SDS), when the diffusion cell has been previously equilibrated with known concentrations of this surfactant. The barrier consists of an aqueous dispersion (3% w/v) of a 2:1 by weight serum albumin-lecithin complex. Ordinates as for Fig. 3.

to any specific lecithin-chloramphenicol interaction, other than the already mentioned "trapping" effect.

When a 3% w/v aqueous dispersion of the albumin-lecithin complex is used as the barrier to chloramphenicol permeation and the diffusion cell is previously equilibrated with premicellar concentrations of an ionic surfactant, i.e. sodium dodecyl sulphate (see Fig. 4), a sharp drop in barrier permeability to chloramphenicol, and conversely a rapid increase in permeation lag times, is observed. This change is already evident through the addition of a small amount of surfactant.

A similar effect is obtained with premicellar concentrations of cetyltrimethylammonium bromide, while no significant variation in both permeability and lag time is observed in the presence of a non ionic surfactant, i.e., polysorbate 80.

To find out whether diffusion and/or dialysis measurements were somehow affected by lecithin-surfactant interaction, the experiments were repeated with an aqueous dispersion of lecithin in the presence or absence of surfactant. In both cases, no significant difference could be found either in permeation or equilibrium dialysis measurements.

Whether a change in viscosity has any consequence on the diffusion rate of the permeant might be questioned. No evidence about this could be obtained by viscosity measurements. In fact, while a slight increase in viscosity occurred as lecithin was added to the protein solution, no significant change could be detected following surfactant addition to an aqueous dispersion of the albumin-lecithin complex.

Different investigators have shown that an alteration of biological membranes by surfactants occurred only in the following conditions:

(a) concentrations of surfactant above the critical micelle concentration,

(b) solubilization of phospholipids or proteins and consequent definite loss of structural integrity (Bradford & others, 1964; Small, Bourges & Dervichian, 1966; Levine, McNary & others, 1970; Gibaldi & Grundhofer, 1972a,b; Feldman & others, 1973).

We suggest that a reversible alteration in the membrane structure, and consequently in the membrane permeability, might be easily effected at the molecular level through a reversible dissociation of structural lipoproteins into their constituents, operated by premicellar concentrations of ionic surfactants.

That a mechanism such as that tested in the present model can take place in physiological conditions, remains to be confirmed.

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