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THE EFFECT OF FATTY ACIDS AND SODIUM DODECYL SULFATE ON THE INDUCED OPTICAL ACTIVITY OF SULFAETHIDOLE - BSA COMPLEXES

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The effect of fatty acids and sodium dodecyl sulphate on the binding of benzodiazepines to bovine serum albumin (BSA) has recently been investigated by a circular dichroic (CD) technique (1). The induced CD spectra attributed to the benzodiazepines - BSA complexes, were modified upon addition of the fatty acids and detergent in a manner which suggested to the authors a conformational rather than a displacement phenomenon. These investigations,unfortunately, were carried out at a high drug to protein ratio of 3.8 and a single high concentration of competitor. As several binding sites seem to contribute to the observed induced ellipticity, it is impossible to clearly establish the effect of any competitor on an individual binding site.

In this communication the effect of octanoic, dodecanoic and hexadecanoic acids as well as sodium dodecyl sulphate (SDS) on the induced optical activity generated by the binding of sulfaethidole to a single site on BSA is reported (2,3,4). Figure 1 shows the effect of SDS on

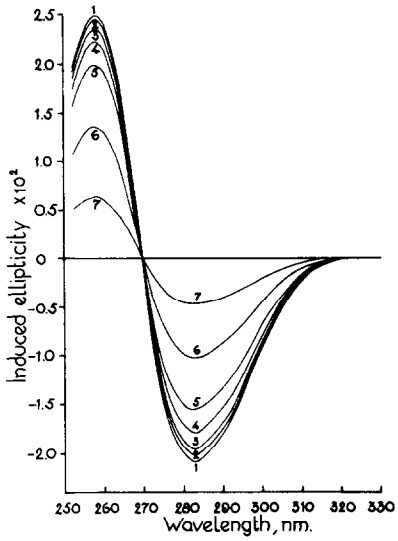

Fig. 1. Corrected induced ellipticity curves for sulfaethidole 2.11 x 10^{-5}M, BSA 1.45 x 10^{-5}M in 1 cm cells, and physiological phosphate buffer of pH 7.4 (Dichrographe III Jobin Yvon, Long Jumeau, France). Experimental methods as in reference 3. SDS concentrations 1) 0, 2) 2 x 10^{-5}M, 3) 3 x 10^{-5}M, 4) 4 x 10^{-5}M, 5) 5 x 10^{-5}M, 6) 7 x 10^{-5}M and 7) 9 x 10^{-5}M.

the induced ellipticity of the sulfaethidole - BSA complex. The positions of the peaks and the isobestic point remain unaffected by the addition of SDS, observations which are consistent with the hypothesis of a direct displacement or a displacement following a conformational change. Similar curves are obtained using octanoic and dodecanoic acids. In the antagonist concentrations of figure 2 and experimental conditions of figure 1 octanoic acid did not disturb the intrinsic optical activity of BSA at wavelenghts between 250 and 300 nm, however

dodecanoic acid (6×10^{-5}M), SDS (5×10^{-5}M) and hexadecanoic acid (1×10^{-5}M) did so at higher concentrations. All induced spectra were corrected for these small effects, these spectral changes were indicative of disturbances in the environment of the aromatic amino acid residues in the BSA. These changes can be compared to the small decrease in magnitude of the intrinsic CD of BSA, on changing the pH from 7.8 to 9.0 and are probably due to the N→B transition as suggested by Müller and Wollert (1). Hexadecanoic acid, which modified the spectra of BSA at the lowest concentrations employed in this report, actually caused an increase in the induced CD spectra of sulfaethidole at concentrations up to 3×10^{-5}M, however at higher concentrations a decrease in signal height was observed as with the other fatty acids. These decreases in signal height of the sulfaethidole - BSA complexes are interpreted as a displacement of the drug by fatty acids and SDS in figure 2. Mean values are calculated

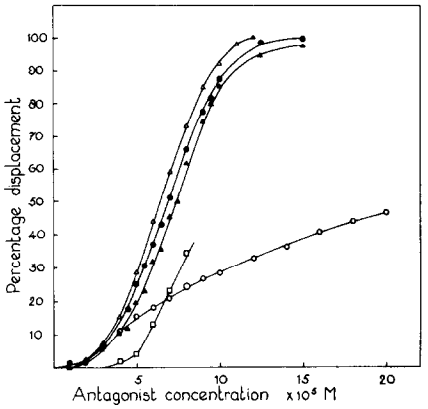

Fig. 2. Decrease in induced ellipticity of sulfaethidole expressed as a percentage displacement. For hexadecanoic (□), octanoic (o), and dodecanoic (Δ) acids, the mean values from the two peaks are shown. For sodium dodecyl sulphate, values for both peaks (● 282 and ▲ 259 nm) are shown.

from the CD peaks at 259 and 282 nm, except in the case of SDS where the displacement was consistently less at the lower wavelength, suggesting that the shape of the induced CD curve changes with the conformational change. The shape of the displacement curves in figure 2 are consistent with the hypothesis (5) that one or two moles of fatty acid or SDS are bound to the BSA before displacement occurs, however only in the case of octanoic acid could a realistic binding constant be calculated using this assumption. The value of 2.2×10^{4}M^{-1} is consistent with the literature value (6) for the second binding site of octanoic acid on BSA.

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