THE BINDING OF SOME ANTI-INFLAMMATORY DRUGS TO ALBUMIN AS MEASURED BY CIRCULAR DICHROISM

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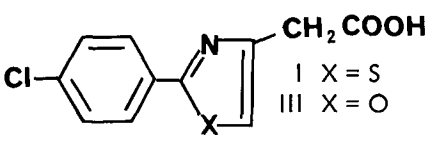
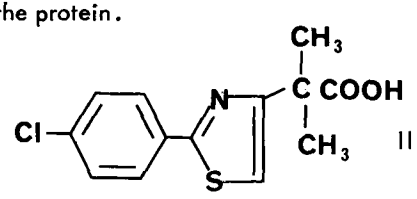
As a class, anti-inflammatory drugs tend to bind strongly to serum albumin. In a number of cases the binding is accompanied by the generation of an extrinsic Cotton effect, which is revealed by comparison of the circular dichroic spectra of the drug and protein taken separately with that of the drug-protein combination (Perrin 1970; Chignell 1973). The change in magnitude of the extrinsic Cotton effect can be used to estimate the parameters of protein binding (Rosen, 1970).

We have studied the change in circular dichroic spectrum arising from the addition of the anti-inflammatory compound [2-(4-chlorophenyl)-4-thiazolyl] acetic acid (I, ICI 54450) to human serum albumin. The difference spectrum is not superimposable on the absorption spectrum of comparable solutions of the pure drug, the normal criterion of an extrinsic effect, but is shifted to shorter wavelengths by 7 nm. However, at 295 nm the maximum of the difference spectrum remains closer to the maximum absorption wavelength of the drug (302nm) than to the nearest absorbing chromophore in the protein.





The change in difference spectrum with concentration of drug has been used to estimate the association constant and numbers of binding sites. The estimate has been made by plotting $\Delta CD/[conc]$ versus [conc] and extrapolating the curve obtained to zero concentration. Using the value so obtained the degree of binding at other concentrations can be calculated (Wheeler, 1974). The Scatchard plot obtained by this method is interpreted as showing two sets of non-interacting binding sites; with $n_1 = 1$, $K_1 = 1.1 \times 10^6 M^{-1}$ and $n_2 = 2.7$, $K_2 = 3.1 \times 10^4 M^{-1}$.

A similar investigation of 2-[2-(4-chlorophenyl)-4-thiazolyl]-2,2-dimethyl acetic acid (II, ICI 55303) showed almost identical binding behaviour, the methyl groups making no effective difference to the values of n and K given above.

The oxazole analog of I (III, ICI 56943) failed to show an extrinsic Cotton effect with our apparatus. This may well be because the absorption maximum of the heterocyclic chromophore is at a lower wavelength (281nm) than that of the thiazole ring. In the presence of protein the total absorption at such lower wavelengths becomes too great to allow meaningful difference spectra to be obtained.

Chignall C.F. (1973). Methods in Pharmacology, Vol.2.
Perrin J.H. (1970). J.Pharm.Sci.,59, 431-448.
Rosen A (1970) Biochem.Pharmacol.,19, 2075-2081.
Wheeler I.F.(1974). Ph.D.Thesis (Lond.).