INTERACTION OF THE ENANTIOMERS OF WARFARIN WITH HUMAN SERUM ALBUMIN, PEPTIDES AND AMINO ACIDS

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The interaction of warfarin and human serum albumin (HSA) may be demonstrated by either quenching the protein fluorescence or by potentiation of the ligand fluorescence (Chignell, 1969). The binding at pH 7.4 of (S)-(-)-warfarin, (R)-(+)-warfarin and (RS)-warfarin to HSA, angiotensin, pentagastrin, tryptophan and tyrosine ethyl ester was examined by a fluorescence titration procedure and the number of binding sites (n) per interactant molecule and the association constants (K) of the complexes calculated by the procedure of Attallah and Lata (1968) (Table 1). Angiotensin, which contains a single tyrosine residue and pentagastrin, which, like HSA, contains a single tryptophan residue, were chosen as model systems.

Table 1. Binding data from fluorescence quenching titrations of (RS)-warfarin and its enantiomers with various interactants.

Interactant P	[P] μM	Warfarin (RS) n	(RS) K*	(R) n	(R) K*	(S) n	(S) K*
HSA ※	11.45	0.93	4.25	0.91	2.50	0.87	5.69
Angiotensin	20.94	1.03	4.69	1.05	2.50	1.03	4.69
Pentagastrin	13.05	0.80	0.90	1.03	0.69	0.97	1.15
Tryptophan	13.10	0.55-0.84	1.38-1.55	0.55-0.85	0.58-1.70	0.58-0.89	1.21-1.33
Tyrosine ethyl ester	49.21	0.50	1.89	0.50	2.97	0.50	1.65

* $(M^{-1}) \times 10^{-5}$ ※ mean values from 9 determinations

(S)-warfarin is significantly more strongly bound to HSA than either (R)-warfarin or (RS)-warfarin. From equilibrium dialysis data, O'Reilly (1969, 1971) found little difference in the binding properties of either enantiomer or the racemate to HSA. The fluorescence quenching titration data (Table 1), however, measures binding at one specific site on the protein molecule, whereas results from equilibrium dialysis include binding at secondary sites.

The results obtained with angiotensin and pentagastrin parallel those obtained with HSA whereas it appears that with the amino acids, two molecules interact with each molecule of warfarin. In all cases there was a hypsochromic shift of the fluorescence emission maximum of the warfarins on binding to the interactant lending credence to the proposal of O'Reilly, Ohms and Motley (1969) that both hydrophobic and hydrogen bonds are involved in the interactions.

Chignell, C.F. (1969). Mol. Pharmacol., 5, 244-252.
Attallah, N.A. and Lata, G.F. (1968). Biochim. Biophys. Acta, 168, 321-333.
O'Reilly, R.A. (1969). J. Clin. Invest., 48, 193.
O'Reilly, R.A. (1971). Mol. Pharmacol., 7, 209-218.
O'Reilly, R.A., Ohms, J.I. and Motley, C.H. (1969). J. Biol. Chem., 244, 1303-1307.