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A circular dichroic investigation of the binding of fenoprofen, 2(3-phenoxyphenyl)-propionic acid, to human serum albumin

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The extrinsic Cotton effects found on the binding of fenoprofen and some of its analogues to human serum albumin have been investigated. The fenoprofen appears to bind hydrophobically to the albumin via the phenyl rings and after dipole interactions of the carbonyl group and the ether oxygen. This binding is non-stereospecific.

Numerous drugs, including flufenamic acid (Chignell, 1969), phenylbutazone (Chignell, 1969; Rosen, 1970), dicoumarol (Chignell, 1969; Perrin & Idsvoog, 1971), warfarin (Perrin & Nelson, 1972), and some long-acting sulphonamides (Kostenbauder, Jawad & others, 1971; Wood & Stewart, 1971) have been reported to become optically active on binding to human serum albumin (HSA). In two instances (Rosen, 1970; Kostenbauder & others, 1971) these extrinsic Cotton effects have been quantitatively interpreted to yield a number of binding sites and binding constants. The technique has shown that the binding of the drugs shows considerable dependence on the species source of the albumin (Chignell, 1969), but little work, other than changes in the hydrophobic portion of the drug molecule, has been done to show which groupings in the drug are essential for the CD signal. Such investigations may give an indication of the nature of the binding sites. In the work reported here, the extrinsic optical activity following the binding to HSA of the non-steroidal anti-inflammatory drug fenoprofen, or its analogues has been investigated in an attempt to find how any induced optical activity arises.

MATERIALS AND METHOD

Fenoprofen, 2(3-phenoxyphenyl)propionic acid, and some analogues were research compounds of Eli Lilly and Co., Indianapolis. The HSA was four times recrystallized material from Nutritional Biochemical Corporation, Cleveland, Ohio. All other chemicals were reagent grade, and deionized water was used throughout. CD spectra were obtained using a 6002 attachment to a Cary 60 spectropolarimeter (Cary Instruments, Monrovia, Ca) with a slit programmed for a half-band width of 1·5 nm. Measurements were made in 5 mm cells using a 0·1M phosphate buffer of pH 5·0. An albumin concentration of $7{\cdot}25 \times 10^{-5}$M was used throughout. The solutions were scanned until the dynode voltage reached 0·4 kV. All induced CD curves became negative at lower wavelengths; however, the high dynode voltage and the increasing intrinsic ellipticity of the albumin did not permit detailed investigations at these wavelengths.

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Table 1. *Characteristics of induced CD curves following the binding of fenoprofen analogues to 7·25 × 10⁵M HSA.*

Drug	Concentration of drug M × 10^3	Peak characteristics: Wavelength nm	Peak characteristics: Observed ellipticity × 10^2	Peak characteristics: Location of shoulder (nm)
(±)-2-(3-Phenoxyphenyl)propionic acid	1·03	282	1·62	276
(+)-2-(3-Phenoxyphenyl)propionic acid	1·03	282	1·66	276
(−)-2-(3-Phenoxyphenyl)propionic acid	1·03	282	1·62	276
(±)-2-(4-Phenoxyphenyl)propionic acid	1·23	282	2·13	276
(±)-2-(2-Phenoxyphenyl)propionic acid	1·23	285	−0·14	290 (neg)
(±)-2-(3-Phenoxyphenyl)propionamide	saturated	281	2·1	—
Ethyl-(±)-2-(3-phenoxyphenyl)propionate	saturated	280	1·47	275
(±)-2-(3-Phenoxyphenyl)acetic acid ..	1·31	282	1·71	276
(±)-2-(3-Phenylthiophenyl)acetic acid ..	1·23	302	1·14	295
(±)-2-(3-Anilinophenyl)acetic acid ..	1·31	320	−0·24	—
(±)-2-(3-Phenylphenyl)acetic acid ..	1·36	292	0·12	—
(±)-2-(3-Phenoxyphenyl)propylamine ..	1·14	—	—	—
(±)-2-(3-Phenoxyphenyl)propanol ..	saturated	—	—	—
(±)-2-(4-Benzylphenyl)acetic acid ..	1·32	—	—	—
(±)-2-(3-Hydroxyphenyl)propionic acid	1·70	—	—	—
(±)-2-(3-Methoxyphenyl)propionic acid	1·66	—	—	—

RESULTS AND DISCUSSION

Initial investigations showed that the dynode voltage was lower and the spectra better defined at pH values of 4·0 and 5·0 than at 7·4. These findings and the observations of Dr. A. Rubin that the binding of fenoprofen to HSA appears to be pH-independent between pH 4 to 10, led to the use pH 5·0 for the investigations. Drug-to-protein ratios of greater than 14 to 1 (Table 1) were used. With the sparingly soluble amide, alcohol and ethyl ester, the concentrations of drug were not known, the solutions being prepared by saturating the albumin solution with drug. At these large drug-to-protein ratios, the signal magnitude was independent of concentration over a small concentration range (±15%), suggesting that the binding sites capable of inducing optical activity were virtually saturated with drug.

Fig. 1 shows the optical activity induced into racemic and (+)- and (−)-fenoprofen; the small inherent optical activity of the enantiomers has been subtracted from the

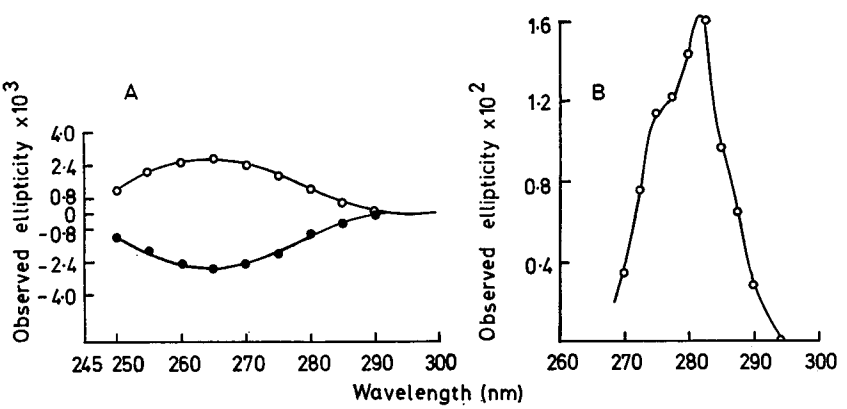

FIG. 1. CD curves for the binding of fenoprofen to 7·25 × 10^{-5}M HSA (drug concentrations in Table 1). (A) Intrinsic optical activity of (+)- and (—)-2-(3-phenoxyphenyl)propionic acids. (no albumin present). Concentrations = 1·03 × 10^{-3}M. (B) Induced CD of (±)-, (−)- and (+)-2-(3-phenoxyphenyl) propionic acid. The curves are virtually superimposable.

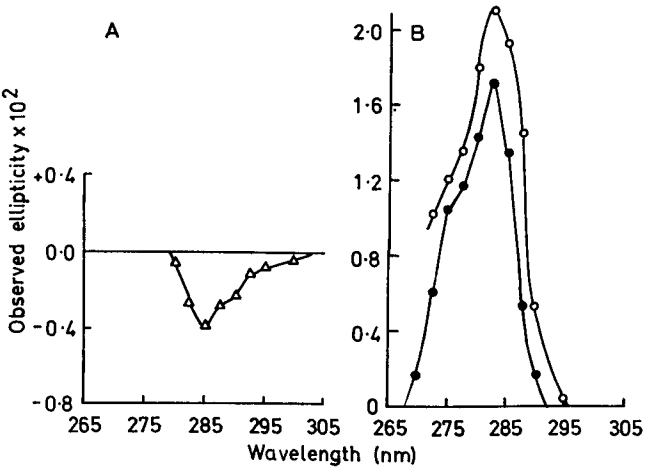

FIG. 2. Induced optical activity of fenoprofen analogues binding to 7·25 × 10^{-5}M HSA. (A) (±)-2-(2-Phenoxyphenyl)propionic acid. (B) ○ (±)-2-(4-Phenoxyphenyl)propionic acid. ● (±)-2-(3-Phenoxyphenyl)acetic acid.

curves. The size and shape of the induced CD curves are similar, suggesting that there is no stereospecificity in the binding. Fig. 2 shows the optical activity induced into *o*- and *p*-(phenoxyphenyl)propionic acid. The shape of the induced curve for the *para*-compound is similar to that of the *meta*-compound but the magnitude of the ellipticity is increased for the *p*-compound, indicating a stronger interaction; however, the *ortho*-compound shows induced ellipticity of opposite sign and of reduced intensity. In the *meta*- and *para*-compounds there is free rotation of the phenyl rings; however, with the *ortho*-compound there is considerable steric hindrance to the rotation. Apparently, this alters the spatial arrangement for the drug-protein interaction and the complex is held rigidly in the region near the chromophore responsible for

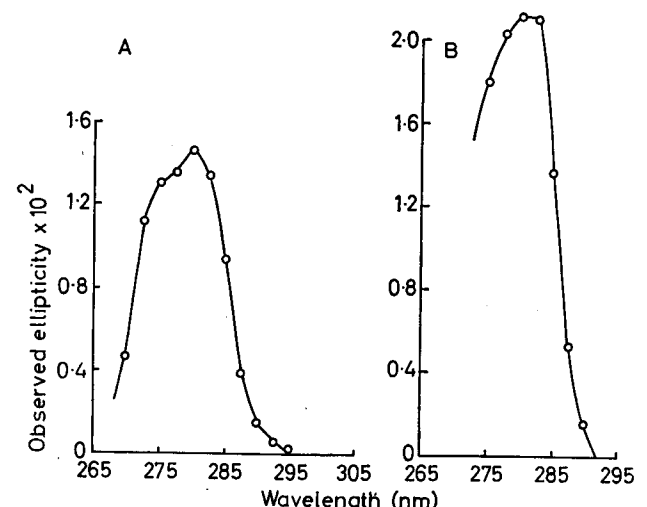

FIG. 3. Induced optical activity of fenoprofen analogues binding to 7·25 × 10^{-5}M HSA. (A) (±)-Ethyl 2-(3-phenoxyphenyl)propionic acid. (B) (±)-2-(3-Phenoxyphenyl)propionamide.

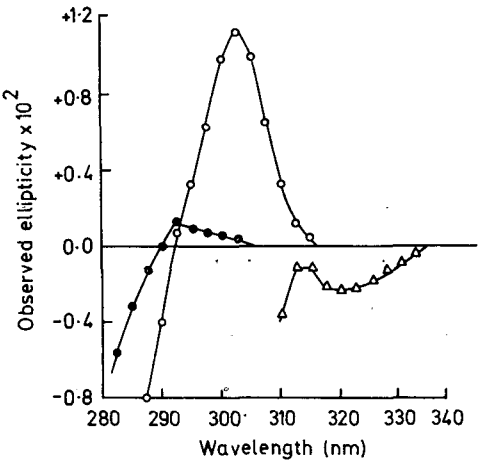

FIG. 4. Induced optical activity of fenoprofen analogues binding to $7{\cdot}25 \times 10^{-5}$M HSA. △ (±)-2-(3-Anilinophenyl)acetic acid. ○ (±)-2-(3-Phenylthiophenyl)acetic acid. ● (±)-2-(3-Phenylphenyl) acetic acid.

negative contributions to the Cotton effects (Chignell, 1969; Schellman, 1968). Fig. 2 also shows that *m*-(phenoxyphenyl)acetic acid has similar optical activity induced into it, as does its propionic analogue. In Fig. 3 the optical activity induced into the ester and the amide of fenoprofen are seen to be similar to that of fenoprofen, whereas *m*-(phenoxyphenyl)propylamine and *m*-(phenoxyphenyl)propanol showed no measurable induced activity.

It appears that binding of the carbonyl grouping to the albumin is essential for the induced activity; this is in agreement with the observed pH independence of the binding. *m*-(Hydroxyphenyl)propionic acid and *m*-(methoxyphenyl)propionic acid show little or no measurable induced activity, and it may be necessary to have two aromatic rings hydrophobically bound to the albumin to give the necessary rigidity for induced optical activity. In an attempt to elucidate the role of the ether oxygen on binding, the compounds shown in Fig. 4 were investigated. *m*-(Phenylthiophenyl)acetic acid gave CD characteristics similar to the phenoxyphenyl compound, the peak wavelength being shifted to a higher wavelength in accordance with the ultraviolet curves absorption spectra. Both the sulphur and the oxygen have non-bonded electron pairs, as does the nitrogen of *m*-(anilinophenyl)acetic acid; however, in the latter case the CD curve is inverted. Possibly the hydrogen of the imido-grouping binds to a negatively charged group in the protein, giving the complex a spatial arrangement different from that which obtains with the preceding two compounds where the sulphur or the oxygen may accept a proton from the protein. The *p*-(benzylphenyl)acetic acid repeatedly gave a very small negative curve whereas the *m*-(phenylphenyl)propionic acid gave a greatly diminished but positive induced CD curve. In investigations of diluted solutions down to 200 nm, there was no change in the intrinsic CD curve of the albumin in the presence of any of the drugs, suggesting that the protein remained in its predominantly α-helical conformation. In conclusion, it appears that the induced optical activity found on binding the anti-inflammatory drug, fenoprofen, to HSA arises as a result of hydrophobic binding of the aromatic rings and hydrogen bonding of the carbonyl and ether oxygen to the HSA.

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