J. Pharm. Pharmacol. 1981, 33: 125–127
Communicated September 8, 1980

0022–3573/81/020125–03 $2.50/0

On the pH dependence of binding of berberine to DNA

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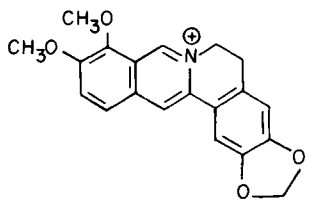
Berberine is a yellow diisoquinoline derivative containing a quaternary nitrogen with a pK_a of about 15. The non-aromatic six-membered ring renders the polycyclic system slightly buckled. Nevertheless, physicochemical studies indicate that the alkaloid can bind to DNA by intercalation (Hahn & Ciak 1974; Krey & Hahn 1969).

Berberine has long been known to have antibacterial and antiprotozoal properties. It can also convert yeasts to respiratory-deficient mutants as a result of its mitochondrial mutagenic action (Hahn & Ciak 1974). A



Berberinium ion

recent study showed its inhibition of DNA, RNA, protein and lipid biosynthesis as well as oxidation of glucose to CO_2 in S180 tumour cells in vitro (Creasy 1979).

On binding of berberine to DNA around neutral pH, bathochromic, hypo- and hyperchromic shifts are observed in the two long-wavelength bands of the berberine spectrum with well-defined isosbestic points (Hahn & Krey 1971). Also, fluorescence of the drug can be enhanced by its interaction with double-stranded and heat-denatured DNA and RNA (Yamagishi 1962). These studies cover only a short pH range around neutrality, which is also the pH region commonly used in the more extensively studied systems of DNA binding to quinoline and acridine drugs (Blake & Peacocke 1968). However, berberine has a structure which is invariant over a wide pH range and should therefore exhibit no u.v.-visible spectral change in this pH region. Thus, unlike many other better investigated drugs whose spectra are dependent on the hydrogen ion concentration, a study of the pH-dependence of berberine binding to DNA by u.v.-visible spectroscopy should be more simply analysed because certain spectral changes would only reflect the berberine environment and mode of binding without the need to consider such complications as the contribution of various drug species involved in the binding.

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Berberine and calf thymus DNA were obtained from Sigma. The DNA (dissolved in 5×10^{-2} M sodium chloride and 5×10^{-2} M sodium citrate, pH 7·0) in a beaker was sonicated at 20 kHz for 15 min before use. This was done to reduce molecular weight with consequent minimization of possible artifacts arising from light scattering, viscosity and optical anisotropy of sample solution. DNA concentration was calculated from the average molar extinction coefficient of 6600 at 259 nm for the bases at pH 7·0. A Beckman Acta V spectrophotometer with thermostatted cell holders and an Aminco Bowman SPF spectrofluorometer with an ellipsoid focusing mirror for the light source were used. A buffer ionic strength of 0·01 was maintained for aqueous solutions throughout; the buffers were chloroacetic acid for pH 2·5 to 3·5; acetic acid pH 4·0 to 5·0; phosphoric acid, pH around 7·0 and tris-(hydroxymethyl)aminomethane for pH from 8·0 to 8·8 and boric acid for pH 9·5 and above (Perrin 1963). Hellma quartz two-chambered cells (pathlength 0·4375 cm/chamber) were used for difference spectroscopic studies.

As expected from the berberine structure, its u.v.-visible spectrum remained invariant from pH 2·3 to 9·5. Over the pH 4·0–9·5 range, the spectral shape from 400 to 550 nm of the completely bound drug was also very similar (Fig. 1). This near identity was also shown by the difference spectra in the ultraviolet. Each series of

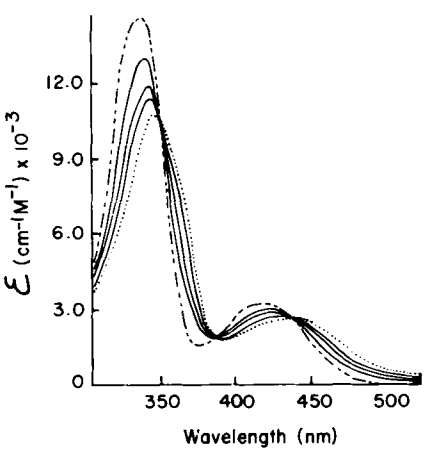

FIG. 1. Long wavelength spectra of berberine (— - - —) and berberine fully bound to DNA (· · · · · · · ·) at pH 7·0, drug concentration of $3·4 \times 10^{-5}$ M and P/D = 11/1. These two curves are representative of the two kinds of spectra from pH 4·0 to 9·5. The spectra in between are typical of DNA titration of berberine only at pH 7·0.

binding curves (obtained in the presence of different concentrations of DNA) showed the same isosbestic points at 353, 384 and 438 nm. The molar extinction coefficients of the two longer-wavelength peaks at extreme pH's were more different than those reported above. It is known that the DNA by itself undergoes structural changes in going from the neutral pH range to the two extremes as indicated by absorbance increases at 260 nm at 25 °C. Also the melting temperature (Tm) of DNA varied with pH, being maximum (72 °C) at about pH 8·5 and 37·5 °C at pH 9·5 and 35 °C at pH 4·0. Berberine stabilized DNA against heat denaturation to a similar extent ($\approx 2°$ to 4 °C) from pH 4·0 to 9·5 (Fig. 2). As a comparison, chloroquine and quinacrine behaved differently in this respect. The molar extinction coefficients of the fully bound berberine (DNA/drug (P/D) of 11 : 1) at 345 nm as obtained from the Linear Least Squares Analysis of the Benesi-Hilderbrand plot (Benesi & Hilderbrand 1949) was found to be 18 300 ± 500 M^{-1} cm^{-1} for pH 4·0, pH 7·0 and 9·6 (cf Davidson et al 1977). All these indicate an identical or extremely similar berberine binding mode (probably intercalation) and environment over this pH range. At pH 3·7, some DNA bases are protonated (Panijpan 1979) and the double helix is ready to fall apart (Tm = 28 °C), berberine with its positive charge could still slightly strengthen the structure against thermal denaturation. We found that chloroquine could compete with this binding.

The fluorescence spectra of bound berberine in the presence of DNA showed the excitation peak at 365 nm and emission peak at 540 nm. The enhancement in fluorescence was always many times that of the free berberine fluorescence which, at the concentration of drug used and our instrument sensitivity setting for this work, could hardly be detected. The fluorescence enhancement on increase of DNA concentration varied with pH (Fig. 3). The enhancement was greatest at pH 3·7 and the increase did not plateau until the P/D ratio was about 110 to 1. The u.v.-visible spectrophotometric change clearly flattens at about P/D of 10:1 at this and other pH's. This observation should make it possible and desirable to detect minute amounts ($\leqslant 10^{-6}$ M) of berberine using fluorescence enhancement by DNA at this low pH. A spectrofluorometric detection of DNA and polynucleotides by berberine is feasible (Yamagishi 1962); this would also be better at pH 3·7.

Indeed such fluorescence enhancement was observed at pH 2·6 and pH 11·5 where u.v.-visible difference spectroscopic magnitudes were extremely small. The above should indicate possible binding of the drug to DNA, albeit less strongly than at more neutral pH's. Heat-denatured DNA also exhibited fluorescence enhancement with berberine. That the enhancement is not due to binding between berberine and the phosphates of DNA was shown by the lack of any fluorescence spectral changes in berberine on addition of 10^{-5} M polyphosphoric acid at pH 3·7 or lower. Pyridine at 10^{-3} M and all four nucleoside triphosphate precursors of DNA at 10^{-4} M also did not affect the berberine (10^{-5} to 10^{-6} M) fluorescence nor its visible spectrum significantly. On the other hand 50% methanol and ethanol enhanced berberine fluorescence, but not to the same extent as DNA at P/D of 110:1 and the u.v.-visible spectrum of berberine did not show the same shifts as when bound to DNA. The absorbance of berberine over

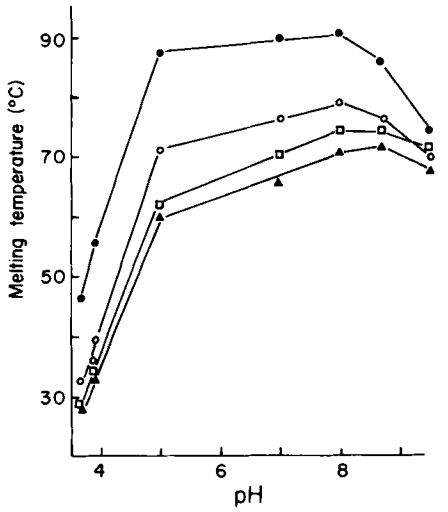

FIG. 2. Melting temperature vs pH profile of DNA (▲——▲), DNA bound berberine (□——□), chloroquine (○——○) and quinacrine (●——●). Drug concentration = $1·5 \times 10^{-5}$M, P/D ratio = 11/1.

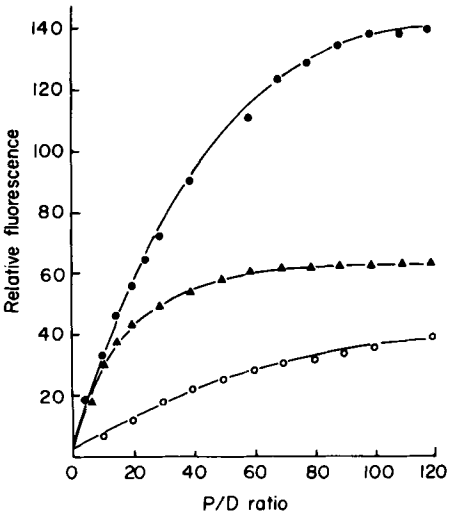

FIG. 3. Fluorescence enhancement of berberine by DNA at pH 3·7 (●——●) pH 8·0 (▲——▲) and pH 11·7 (○——○). Berberine concentration was $3·0 \times 10^{-6}$M. The fluorescence vs P/D ratio profile was similar for pH 5·0 to about 9·6. Excitation at 365 nm and emission at 540 nm.

the concentrations used and the DNA-berberine mixture (P/D = 11/1) at various concentrations up to 10^{-5} M strictly followed Beer's Law. Thus DNA-berberine fluorescence enhancement at pH 3·7 possibly reflects high quantum yield complex formation with the DNA bases, which are still closely arranged, providing the relatively hydrophobic environment for interaction (Love et al 1978). Many drugs have been known to bind preferentially to regions in the DNA molecule (Pack & Loew 1978; Weisblum & de Haseth 1972; Jorgenson et al 1978). On interaction with the drugs, regions with different binding affinity may give different fluorescence quantum yields. Perhaps, as DNA concentration increases, berberine migrates from lower affinity binding sites to higher affinity binding sites with a consequent enhancement in fluorescence but no significant change in the u.v.-visible spectra. When the P/D ratio reaches 110/1 all drug molecules have occupied the high affinity sites. From our studies, chloroquine and quinacrine binding to DNA did not lead to such high fluorescence enhancement over pH 2·5 to 11·5.

This work was supported by the National Research Council of Thailand and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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LETTER TO THE EDITOR

J. Pharm. Pharmacol. 1981, 33: 127–128
Communicated June 27, 1980

0022–3573/81/020127–02 $2.50/0

Relationship between inhibition of prostaglandin production and gastric mucosal damage induced by anti-inflammatory drugs may depend on type of drugs and species

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Recently, it was reported that inhibition of prostacylin (PGI_2) production may be related to the appearance of gastric lesions induced by non-steroid anti-inflammatory (NASI) drugs in rats (Whittle et al 1980). In terms of quantitative relevance to studies in man it is PGE_2 and not PGI_2 which is the main prostaglandin in the human gastric mucosa (Peskar et al 1980) and, as well, in pig (Rainsford & Peskar 1979), dog (Skoglund et al 1980) and cat (S. J. Kontureck and R. Gryglewski, personal communication). However, prostacyclin is the predominant prostaglandin in the rat mucosa, so that studies in this species might give unique results of limited relevance to the situation in man. Moreover, the fact that PGI_2 is derived mainly from vascular tissues and its production and/or functions may be regulated by PGE_2 (Tomasi et al 1978; Gordon et al 1979) indicates that the ratio of both these prostaglandins may be more important in determining the relevance of prostaglandin inhibition in the development of gastric mucosal damage by NSAI drugs.

We have, therefore, compared the gastric ulcerogenic effects of NSAI drugs with their effects on the levels of PGE_2 and 6-keto $PGF_{1\alpha}$ (stable PGI_2 hydration product) in the gastric mucosa and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (-k-H_2-$PGF_{2\alpha}$, the $PGF_{2\alpha}$ metabolite) in plasma of pigs. This species has (i) a stomach resembling that of man in respect of morphology and function (c.f. rat), and (ii) similar responses in the gastrointestinal mucosa to a variety of NSAI drugs as observed clinically in man (Rainsford & Peskar 1979). The pigs were dosed orally for 10 days with the drugs using procedures as described previously (Rainsford 1978) (see also footnotes to the Figure). The prostaglandin content of

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