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The anti-mutagenic and antioxidant effects of bile pigments in the Ames *Salmonella* test

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Abstract

The aim of this study was to explore the potential pro- and anti-mutagenic effects of endogenous bile pigments unconjugated bilirubin (BR), biliverdin (BV) and a synthetic, water soluble conjugate, bilirubin ditaurate (BRT) in the Ames *Salmonella* test. The bile pigments were tested over a wide concentration range (0.01–2 μmol/plate) in the presence of three bacterial strains (TA98, TA100, TA102). A variety of mutagens including benzo[α]pyrene (B[α]P), 2,4,7 trinitrofluorenone (TNFone), 2-aminofluorene (2-AF), sodium azide (NaN_3) and tertiary-butyl hydroperoxide (*t*-BuOOH), were used to promote the formation of mutant revertants. Tests were conducted with (B[α]P, 2-AF, *t*-BuOOH) and without (TNFone, NaN_3, *t*-BuOOH) metabolic activation incorporating the addition of the microsomal liver preparation, S9. The bile pigments alone did not induce mutagenicity in any of the strains tested ($p > 0.05$). Anti-mutagenic effects of the bile pigments were observed in the presence of all mutagens except for NaN_3 and the anti-mutagenic effects appeared independent of the strain tested. For TNFone induced genotoxicity, the order of effectiveness was BR ≥ BRT > BV. However, the order was BV ≥ BRT ≥ BR for 2-AF. Antioxidant testing in the TA102 strain revealed bile pigments could effectively inhibit the genotoxic effect of *t*-BuOOH induced oxidative stress. The apparent antioxidant and anti-mutagenic behaviour of bile pigments further suggests their presence in biological systems is of possible physiological importance.

Keywords: Bile pigment; Bilirubin; Biliverdin; Bilirubin ditaurate; Antioxidant; Antimutagen; Ames test; *Salmonella*

1. Introduction

Bile pigments, unconjugated bilirubin (BR) and biliverdin (BV) are tetrapyrrolic, dicarboxylic acids belonging to the '*porphyrin*' class of molecules [1] (Figs. 1 and 2). Large quantities of bile pigments are formed in humans on a daily basis and the excretion of bile pigments in the faeces and urine is estimated at 300 mg per day [2]. Bilirubin and biliverdin are potent antioxidants, and as such a possible physiological role for them has been postulated [3]. Specifically, they have been found to protect lipids [4–9] and proteins [10–12] from oxidation. Additional studies also

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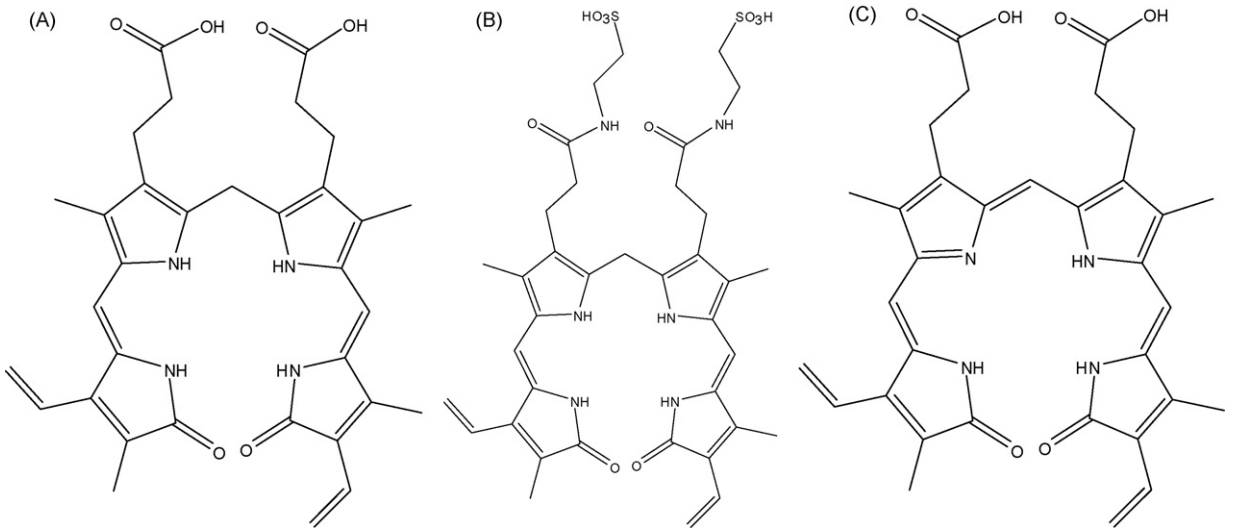

Fig. 1. Two-dimensional, planar structures of unconjugated bilirubin (A), bilirubin ditaurate (B) and unconjugated biliverdin (C).

suggest that bile pigments possess anti-complement [13,14], anti-inflammatory [15–17], anti-viral [18,19], anti-apoptotic [20] and anti-mutagenic [21–30] properties, fuelling the debate that bile pigments are not simply by-products of haem catabolism, but physiologically important molecules.

The Ames *Salmonella* test [31] has been a popular test model for studying the anti-mutagenic effects of bile

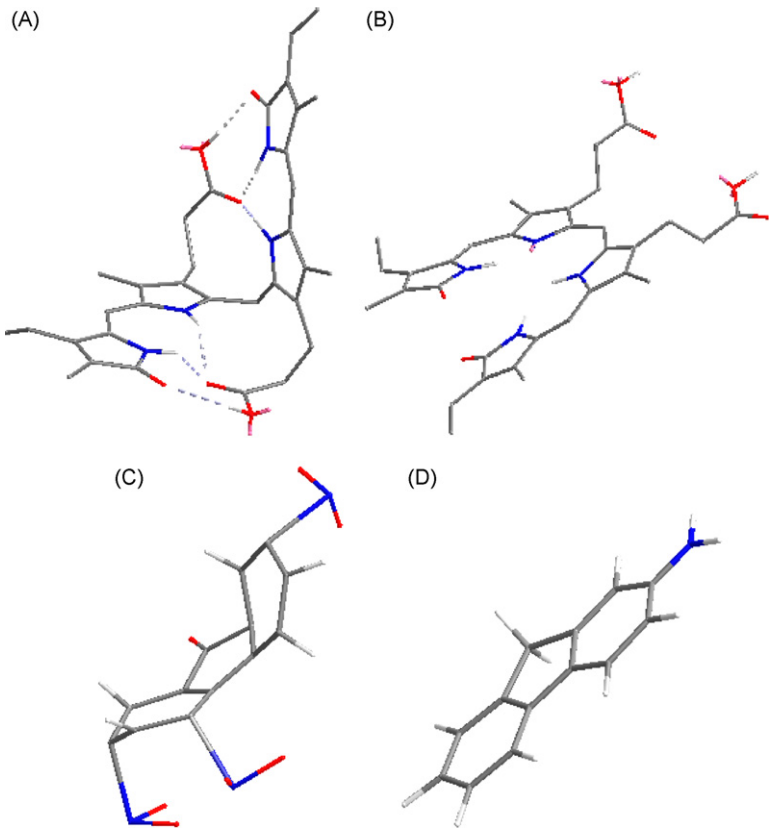

Fig. 2. Three-dimensional, energy minimised (MM2) structures of unconjugated bilirubin (A), unconjugated biliverdin (B), 2,4,7 trinitrofluorenone (C) and 2-aminofluorene (D).