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Research Article

# Curcumin Inhibits Protein Tyrosine Phosphatase 1B and Related Redox Sensitive Tyrosine Phosphatases

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## Abstract

Dietary supplements are actively promoted for disease prevention and control. Consumers may make the incorrect assumption that these products are inherently safe because they are "natural". However, phytochemicals in such supplements can have molecular effects that raise safety concerns. We investigated the molecular toxicology of curcumin, a dietary supplement advocated for chemoprevention of intestinal and colorectal cancers. Because of its intrinsic potential as an electrophile and its symmetrical methoxyphenol substituents we hypothesized that curcumin would inhibit redox sensitive tyrosine phosphatases, typified by protein tyrosine phosphatase 1B. Our results validate this hypothesis. Since inhibition of protein tyrosine phosphatase 1B in cells would suppress a process that restrains growth factor signaling pathways we speculate that curcumin imposes risks that are inseparable from its still unproven benefits in cancer prevention.

***Keywords:*** *Protein Tyrosine Phosphatase 1B; Curcumin*

## Abbreviations

EGFR: Epidermal Growth Factor Receptor; FDA, Food and Drug Administration; LAR: Leukocyte Common Antigen Related; PTP: Protein Tyrosine Phosphatase; SHP: Src Homology 2 Domain Containing Phosphatase; TP: Tyrosine Phosphatase

## Introduction

Turmeric, a spice from the plant Curcuma longa, is used as a traditional medicine for gastric and inflammatory ailments [1]. A group of phenolic compounds, typified by curcumin, are the principal active ingredients in turmeric. Curcumin dietary supplements are promoted as a naturopathic remedy, in parallel with growing, popular interest in complementary and alternative medicine for disease control. An epidemiological association of dietary turmeric with a low incidence of large and small bowel cancer in India [2] has been used to advocate for curcumin use in cancer chemoprevention.

Dietary supplements are not approved by the US FDA for treatment or prevention of any disease. Nevertheless, they are often advertised for medical use, and many cancer patients, cancer survivors, and individuals pre-disposed to cancer take dietary supplements for prevention, treatment, or supportive care [3]. Determining the molecular mechanisms of action of these supplements is necessary to define their pharmacology and toxicology and, thereby maintain a balanced perspective on the safety and efficacy of phytochemicals in cancer control [4].

Curcumin has chemical traits that may compromise its safe and effective use for medical purposes [5-7]. Nelson., *et al*. have termed curcumin a 'universal invalid panacea,' which seems apt considering the scope of its reported benefits, without credible corresponding validation from well-designed clinical trials [5-7]. Curcumin contains two, symmetrical methoxy-phenol substituents and an α, β-unsaturated ketone, which can covalently modify redox sensitive proteins [8], especially at nucleophilic cysteine residues that are essential for their function. Accordingly, we hypothesized that curcumin would inhibit redox sensitive protein tyrosine phosphatases, and thereby confer risks that are inseparable from its potential benefits in cancer prevention. Our results support this hypothesis.

## Materials and Methods

We used the following recombinant tyrosine phosphatase enzymes (Enzo Biochemical): PTP1B, residues 1-322, CD45, LAR, T cell TP, SHP-1, SHP-2. The tyrosine phosphatases we investigated share some homology in the region surrounding the catalytically active site of PTP1B. As substrates to quantify enzyme activity we used the phosphotyrosine peptide which corresponds to the amino acid residues 988-998pY992 of the EGF receptor, and the IR5 phosphotyrosine peptide, which contains the amino acids 1142-1153pY1146 of the insulin receptor β subunit. The enzyme assay buffer was 50 mM HEPES pH 7.2, 1 mM EDTA, 0.5% dithiothreitol, 0.05% NP-40 non-ionic detergent and bovine serum albumin, 0.2 mg/ml. We established that the PTP1B recombinant enzyme in this buffer retained 99 ± 1% of its initial activity when standing for 15 minutes at 4°C.

We added curcumin dissolved in 5 µL methanol to 200 µL polyethylene centrifuge tubes and evaporated the solvent. We then added 20 µL of assay buffer containing PTB1B (5 ng) and incubated for 1 minute at 4°C, followed by 10 µL of assay buffer containing 3 nanomoles of phosphotyrosine peptide substrate and incubated at 4°C for another 2 minutes. The concentrations of curcumin in the assay were between 0-30 µM and the final concentration of peptide substrate was 100 µM. The phosphatase reaction was quenched by transferring the entire 30 µL reaction mixture to microtiter plate wells containing 100 µL of a solution of Biomol® Green (malachite green). This reagent forms a colored complex with inorganic phosphate. Absorbance at 615 nm reflects enzymatic activity. Color developed for 25 - 30 minutes, and absorbance was measured using a Spectra Max M2 spectrophotometer (Molecular Devices, Sunnyvale, CA). Data depict the mean ± standard error of the mean (n = 3 - 10). Inorganic phosphate standards were measured periodically. Some assays were conducted to compare the effect of curcumin on PTP1B activity with two different phosphotyrosine peptide substrates, and to compare different tyrosine phosphatases.

## Results

Figure 1 shows that curcumin inhibits the catalytic activity of PTP1B and a few representative tyrosine phosphatases in a dose-dependent manner. The rank order of inhibition based on the $IC_{50}$ shown in figure 1 was: T cell PT≅ PTP1B > LAR > CD45>>SHP1 & 2 (data not shown). The two sets of data showing inhibition of PTP1B represent results from experiments using two different phosphotyrosine peptide substrates derived from the EGF receptor (□) and the insulin receptor β subunit (□). At concentrations > 50 µM curcumin is insoluble in the assay buffer; thus, dose response curves cannot extend beyond the values shown.

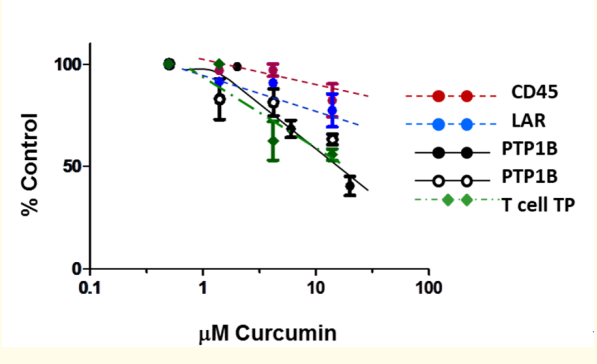
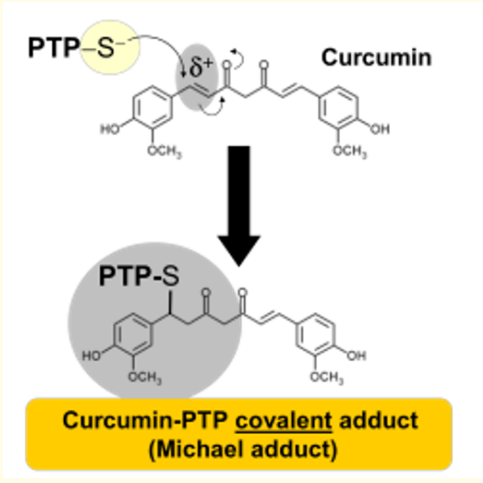
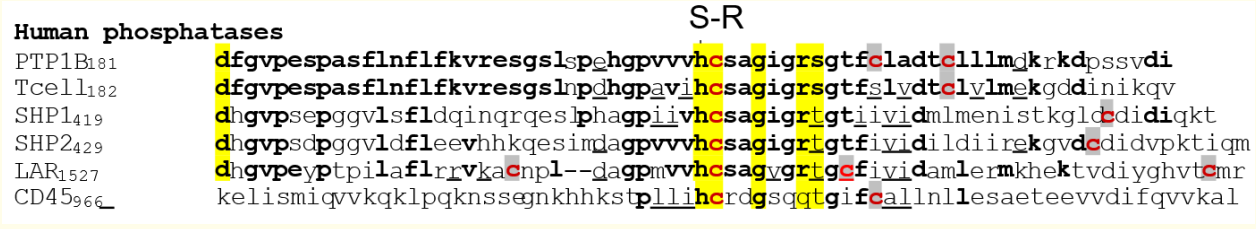



***Figure 1:*** *Curcumin inhibits protein tyrosine phosphatase 1B and related redox sensitive tyrosinephosphatase enzymes in a concentration-dependent manner.*

Figure 2 shows how our results relate to the primary amino acid sequence of the tyrosine phosphatases and a proposed mechanism for the curcumin effect. Tyrosine phosphatase family members all have the same catalytic site signature. We speculate, based on precedents with a related member of the tyrosine phosphatase super family [9], that curcumin modifies the catalytic cysteine of PTP1B. The results suggest that overall homology (T cell TP and PTP1B) in the region harboring the catalytic site strongly influences susceptibility to curcumin. When the overall homology is weaker, the data support the hypothesis that cysteine on the carboxy terminal side, e.g. grtgcf residues in LAR, has an influence. This is under investigation.



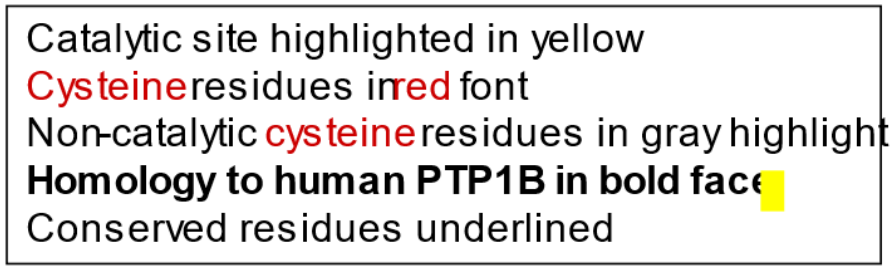




***Figure 2:*** *Hypothesis for molecular mechanism of curcumin inhibition of protein tyrosine phosphatase 1B and related redox sensitive tyrosine phosphatase enzymes.*

## Discussion

Diet and inflammation modify colon cancer risk in complex ways [10]. Diet or dietary supplements can be beneficial, neutral, or harmful, depending on i) the chemicals involved; ii) how these chemicals act on epithelial, mesenchymal and immune cells; or iii) how they act on inflammation in the gut. In cancer control, beneficial health effects attributed to diets are seldom reproduced by administration of supplements containing either a single component or a few components of that diet [10,11]. Likewise, population-based evidence that

attributes beneficial effects to diets or ingredients in diets must be sufficiently rigorous to exclude confounding variables and alternate hypotheses. Curcumin typifies this complexity. On the "pro" side, epidemiological studies show that people in Asian countries, who eat diets rich in turmeric (curcumin), have low rates of colorectal cancer [1]. This is attributed to antioxidant and anti-inflammatory effects of curcumin. On the "con" side, curcumin impairs the p53 tumor suppressor protein [6]. Impairment of p53 structure-function occurs in over 50% of solid tumors, and p53 impairment is associated with colon tumor progression and a poor prognosis. Poor bioavailability of curcumin limits its systemic effects; however, the cells lining the gut can be exposed to levels of curcumin that warrant concern.

Protein tyrosine phosphatase 1B restrains signaling by several growth factors, e.g. epidermal growth factor, insulin, insulin-like growth factors [12]. By inhibiting PTP1B enzymatic activity curcumin may have an impact on growth factor signaling pathways. Removal of the tyrosine phosphatase restraint on tyrosine kinase signaling could be beneficial in some circumstances, e.g. ulcer healing. Conversely it could be detrimental in others, e.g. tumor progression in a pre-malignant colorectal adenoma, or liver tumors [13]. It is noteworthy that LI., *et al*. [14] have reported that curcumin also inhibits the expression of PTP1B mRNA and protein in the liver of rats.

## Conclusion

The Institute of Medicine and the National Research Council have issued a report to help define scientific principles for risk assessment. This report states: "...attention should focus on signals which indicate that a serious health problem may result due to ingestion of a dietary supplement ingredient". The report stresses that these signals may emerge from four categories of information: 1) human data, 2) animal studies, 3) *in vitro* experiments, and 4) information on related substances. Our *in vitro* results pertain to an objective evaluation of the benefits and risks of curcumin in complementary and alternative medicine.

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