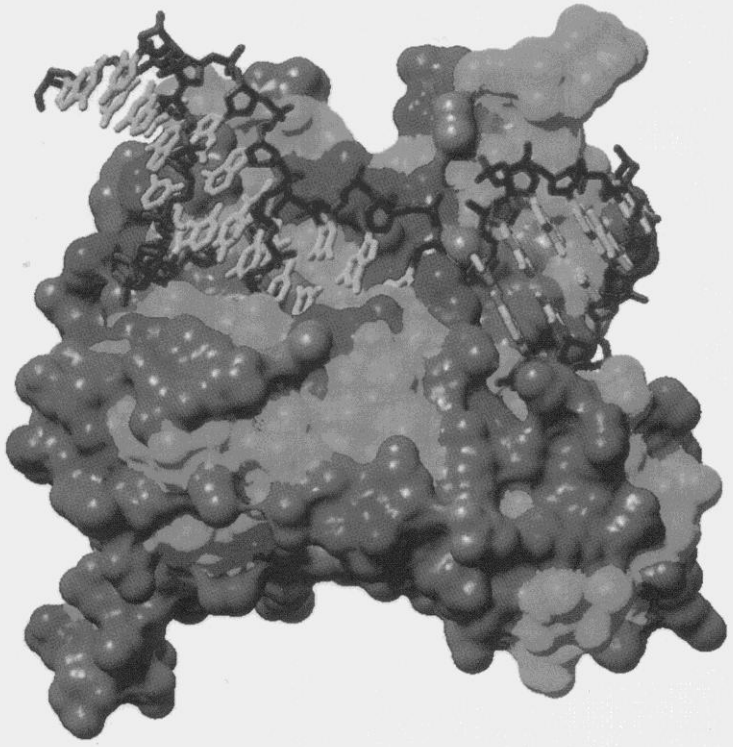
IJB the Italian Journal of Biochemistry

Vol. 53 n. 3 September 2004

Special Issue:
SIB 2004
Riccione, September 28 - October 1, 2004



Official Organ of the Italian Society of Biochemistry and Molecular Biology

Trimestrale - Sped. in a. p. - 45% - art. 2 comma 20/b legge 662/96 - Filiale di Milano - ISSN: 0021-2938

BETANIN INHIBITS MYELOPEROXIDASE/NITRITE-MEDIATED PEROXIDATION OF HUMAN LOW-DENSITY LIPOPROTEIN

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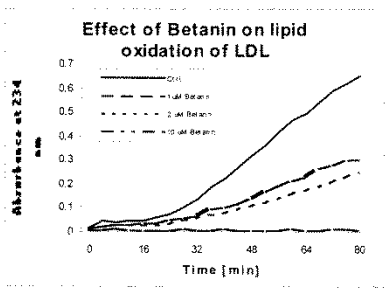
INTRODUCTION: Betanin, the betalain red pigment occurring in the Cariophillalae order plants, including cactus pear, has recently been reported to posses reducing properties and to behave as lipoperoxyl radical-scavenger *in vitro* (1). In addition, this phytochemical is bioavailable, accumulates in human LDL after ingestion of cactus pear fruits, and is able to protect LDL against copper-induced oxidation *in vitro* (2,3). Myeloperoxidase (MPO) has been implicated in the *in vivo* LDL modification and atherogenesis (4). The enzyme, in the presence of nitrite, generates two powerful oxidizing agents, the tyrosyl radical and the nitrosyl one, both of which promote LDL lipid oxidation (4). Taking all this into account we have decided to investigate weather betanin could counteract MPO/nitrite-induced oxidation of LDL.

MATERIALS AND METHODS: Human MPO was purchased from Calbiochem and Glucose oxidase from Sigma. All other chemical and solvents were purchased from Sigma Aldrich or Merck. Preparation of LDL. LDL was prepared from blood serum of healthy volunteers according to Kleinveld et al. (5) with minor modification and stored in the presence of 4mM EDTA at –80°C. Lipid peroxidation of LDL. Reactions were carried out according to Kostyuk et al (6).

RESULTS: Our results, indicate that betanin is able to inhibit the MPO/nitrite-induced LDL lipid peroxidation, in a dose-dependent manner in the range 1 to 10 μM (Fig.1). We have compared the effectiveness of betanin with that of two well-known physiological antioxidants: α-tocopherol and ascorbic acid. As reported (4), α-tocopherol, the most powerful lipoperoxyl radical-scavenger, is only able to partially protect LDL lipids from oxidation by the MPO/nitrite system and has scarce or no effect on the powerful hydrophilic oxidants generated by nitrite. On the contrary, vitamin C, which is able to scavenge the peroxidase-generated nitrating species, was very efficient in counteracting the MPO/nitrite-sustained lipid peroxidation. Betanin, was even more effective than vitamin C, at inhibiting the oxidative damage to



LDL. The IC_{50} calculated for betanin (1.4 μM) was more than 10-fold lower than that for ascorbic acid (15.6 μM).

Nitrite, an oxidation product of nitric oxide metabolism, and MPO are considered mediators of the LDL oxidation process in vivo. Our study shows that betanin, a phytochemical occurring in the cactus pear fruit, is able to protect LDL, in an experimental set-up of physiological relevance. In addition, the molecule acts at micromolar concentrations, and appears much more effective than ascorbic acid. Our data collectively indicate a favourable modulation of the oxidation process of LDL and may contribute to the supposed beneficial effect of cactus pear fruits (2).

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