ANTIOXIDANT PROPERTIES OF INDOLE-3-PYRUVIC ACID

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1. INTRODUCTION

Oxidative stress is believed to be involved in the pathogenesis of many important degenerative diseases, especially in the Central Nervous System, where oxygen is utilized in very high amount, and scavengers of toxic substances might be insufficient when neurons are over-stimulated or ageing processes accumulate peroxide lipids (Halliwell, 1992).

To counteract oxidative stress, living organisms selected several biomolecules, able to scavenge oxygen free-radicals, or to transform them to less injurious agents: Glutathion, Vitamins C and E, Enzymes (e.g. Superoxide Dismutase and Catalase), and so on. Indole compounds constitute one of the most important classes of substances interacting with toxic oxygen derivatives and, because of their widespread presence in any kind of living cell, they can be considered within the first defense systems developed during evolution. Among the others, Indole-3-pyruvic acid (IPA), the keto-analogue of tryptophan that can be easily obtained from the aminoacid by means of several aromatic aminotransferases or aminoacid oxidases, is arising as a pre-eminent indole in living organisms because it replenishes cells with important biochemicals and behaves like a strong antioxidant.

2. IPA IN THE VEGETAL KINGDOM

Indoles are extensively utilized by plants as "auxins", or growth-inducers, and indole-3-acetic acid (IAA) is recognized as the most important compound of this class. The biochemical pathway of IAA synthesis from tryptophan, including IPA as the central intermediate, has been established in several plants and microorganisms, and enzymes like aromatic aminotransferase and indolpyruvate decarboxylase have been described in details (El-Abyad et al., 1994; Koga et al., 1994), so that this is considered the usual mean utilized by plants for their growth, even if some indole side-chain reactions have been recently proposed as alternate pathways (Normanly et al., 1995).

When plants stop growing, however, IAA production is strongly reduced, and IPA accumulates in senescent leaves up to very high concentrations (Atsumi and Hayashi, 1979).

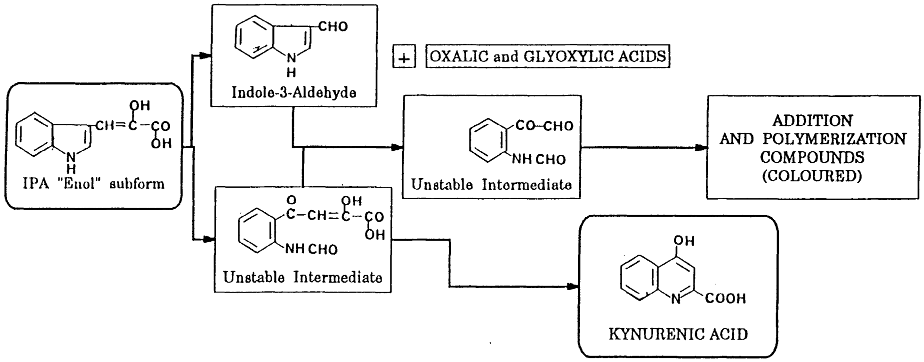

Figure 1. IPA main Pathways after oxygen attack. Development of coloured compounds is believed to prevail in plants, while Kynurenic acid is more important for animal cells.

Bright colours, going from yellow to red and brown, are often observed in dying leaves of higher plants during the autumn: this reflects reactions of IPA with oxygen species (Figure 1), and the same colours are easily obtained in test tubes when IPA is dissolved in aerated solutions in the presence of light. On the other hand IPA, as well as other indoles found in plants, are well recognized as good electron donors, and intense colours develop when they are put in contact with electron acceptors (Hutzinger et al., 1972), suggesting a direct participation also to colour development in flowers.

3. IPA AS A TARGET FOR RADICAL ATTACK

It is believed that the strong antioxidant properties of indoles present in living cells are especially due to a radical attack on carbon atoms in positions 2 and 3, after abstraction of hydrogen from the N-H bond (Lissi et al., 1991). When considering IPA molecule, however, reactivity toward oxygen and oxygen radicals is increased, because in its "enol" configuration the ketoacid has a planar structure, with another double bond in the side chain, conjugated with that present in the indole nucleus.

Some time ago, it has been shown that oxygen attack on IPA induces release of chemiluminescence, attributed to several open-ring species (Zinner et al., 1974). The discovery that oxydation of IPA induces also formation of kynurenic acid both in the presence and absence of enzymatic systems (Russi et al., 1989; Politi et al., 1991), authorizes some conclusions about chemicals formed when IPA reacts with oxygen free-radicals. As described in Fig.1, after an attack on the side-chain, compounds like oxalic and glyoxylic acid are easily found in solutions, while the opening of indole ring by oxygen radicals is followed by formation of unstable compounds, further transformed to kynurenic acid or to addition and polymerization products (most of them coloured).

As a strong free-radical scavenger, IPA was found to inhibit radical damages in abiotic systems as well as in organ homogenates (Politi et al., 1991). Furthermore, the ketoacid showed powerful des-mutagenic properties in the Ames test (Table 1): using non-toxic concentrations of IPA, dramatic reductions in the mutagenicity of 4-Nitroquinoline-N-oxide (4NQO), 2-Nitrofluorene (2NF), Captan (CAP) and Folpet (FOL) were observed. These effects were not slight diminutions in activity, but substantial reductions in the mutagenic potency of the products, recognized as free-radical-forming agents. Instead, no reductions were observed in the mutagenicity of other substances, like Ethylmethanesulphonate, Methylmethanesulphonate or Sodium Azide (data not shown), whose mechanism of toxicity