

MICROSOMAL DEGRANULATION BY ISATIN AND ITS INHIBITION BY ASCORBIC ACID

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CARCINOGENS are known to degranulate rough endoplasmic reticulum both under *in vivo*¹ and *in vitro*² conditions. Measurement of *in vitro* degranulation has been suggested to be a useful technique for determining the carcinogenic potentials of environmental chemicals^{2, 3}. Even anticarcinogens can be detected by assaying the inhibition of degranulation⁴. Isatin (2,3-dioxoindoline) and its derivatives have been reported to exhibit antiviral, antimicrobial and anti-fungal activities⁵. These compounds have also been found to potentiate the analgesic effect of morphine⁵. Studies on green pea stem sections show isatin to elicit auxin (plant growth hormone) like properties⁶. It is now well known that certain plant growth hormones promote the growth of tumours⁷. We studied, therefore, the carcinogenicity of isatin by microsomal degranulation. We report here that isatin is a potential carcinogen but this activity is antagonised by ascorbic acid which is considered to be an anticarcinogen⁴. These studies indicate that carcinogenicity of some chemical compounds in human environment might depend upon the nutritional status of a cell with respect to ascorbic acid.

For *in vivo* studies, female albino rats weighing 200 g each and fed *ad libitum* were divided into three groups and each group was given various oral doses for six consecutive days. To the first group (control) 1 ml of DMSO was given. To the second group 20 mg of isatin dissolved in 1 ml DMSO and to the third group in addition to isatin, ascorbic acid dissolved in 1 ml of distilled water was given as an anticarcinogen. After the last dose, the food was removed and the animals were provided with water *ad libitum*. They were sacrificed after 24 hr and microsomes from their livers were prepared as described previously⁴.

For *in vitro* studies, female albino rats (weighing 150 to 200 g each) were sacrificed and microsomes from their livers were prepared as previously described⁴. Degranulation studies were performed in a total volume of 5 ml, containing 1.4 ml microsomal suspension (5 to 10 mg/ml protein), 0.5 ml NADPH regenerating system⁸ (25 μ mole glucose-6-phosphate, 25 μ mole MgSO₄, 100 μ mole nicotinamide and 0.6 μ mole NADP) or double distilled water, 0.1 ml isatin (20 or 40 μ g/ml final concentration) dissolved in DMSO or DMSO, 0.5 ml of post-microsomal supernatant or ST buffer (0.225 M sucrose and 25 mM Tris at pH 7.4) and 2.5 ml ST buffer. For experiments to study the effect of ascorbic acid on degranulation, 0.1 ml of ascorbic acid (40 μ g/ml final concentration) was also added to the above mixture and the mixtures, as detailed in Table I, were incubated at 20°C for 2 hr in a water bath. The degranulated microsomes or intact vesicles after the above treatments were sedi-

TABLE I
Degranulation of rat liver microsomes by isatin and its protection by ascorbic acid

Composition of incubated mixtures	Per cent loss of RNA	
	RNA/protein basis Mean \pm S.D. ^c n=6	RNA/phospholipid basis Mean \pm S.D. ^c n=6
(a) <i>In vitro</i> experiments		
Microsomes + ST buffer(control)	0.0	0.0
Microsomes + NADPH + DMSO + ST buffer	2.5 \pm 0.5	0.0
Microsomes + isatin (40 μ g/ml) + ST buffer	6.2 \pm 0.4	7.7 \pm 0.3
Microsomes + NADPH + isatin (40 μ g/ml) + ST buffer	37.5 \pm 1.9	45.4 \pm 2.4
Microsomes + NADPH + PMics ^b + isatin (40 μ g/ml) + ST buffer	37.5 \pm 2.1	41.5 \pm 1.8
Microsomes + NADPH + isatin (20 μ g/ml) + ST buffer	31.2 \pm 1.3	35.0 \pm 1.4
Microsomes + NADPH + ascorbic acid (40 μ g/ml) + isatin (40 μ g/ml) + ST buffer	6.2 \pm 0.2	5.1 \pm 0.3
(b) <i>In vivo</i> experiments		
Microsomes from DMSO-treated rats (control)
Microsomes from isatin-treated rats	41.3 \pm 1.6	42.5 \pm 3.1
Microsomes from ascorbic acid + isatin-treated rats	0.0	3.8 \pm 0.4

(a) DMSO stands for dimethyl sulfoxide. (b) PMics stands for post-microsomal supernatant.

(c) For each set two different experiments were performed and each experiment involved assays in triplicate.

mented at 9,000 g for 20 min at 4° C and the washed pellets were resuspended in ST buffer having about 5 mg/ml protein.

Protein, RNA and phospholipid estimations were done according to standard methods⁴.

Experiments conducted under *in vitro* conditions show (Table I) that NADPH and DMSO do not degranulate the reticular membranes. However, isatin caused degranulation around 7% in the absence of NADPH and around 40% in the presence of NADPH. Thus the function of isatin to act as a carcinogen seems to be dependent on NADPH, required for the conversion of a number of carcinogens to their electrophiles by the microsomal hydroxylase system^{2,3}. This conversion has also been reported to be favoured by the presence of smooth membrane vesicles⁵ which are present in our preparations. It may be possible that the -NH group present in isatin is converted to -NOH by the action of microsomal hydroxylase system, thereby producing an active carcinogen. Ascorbic acid has been reported to be antagonistic to the carcinogenic activity of nitrosamine⁶. Our results also demonstrate the anticarcinogenic activity of ascorbic acid under *in vitro* conditions, which corroborates the results of our earlier communication⁴.

Results presented in Table I show that about 42% ribosomal loss is observed in microsomes prepared from livers of animals treated with isatin both on the bases of RNA/protein and RNA/phospholipid ratios. But in the third group of rats ascorbic acid treatment antagonized the effect of isatin and the microsomal degranulation in this group was reduced to negligible levels.

Data reported in this paper show that isatin can act as a carcinogen and ascorbic acid antagonizes that activity.

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LIGNOCLASTIC ACTIVITY OF *ASPERGILLUS CLAVATUS*, *PENICILLIUM MARTENSII* AND *PYTHIUM PROLIFERUM*

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LIGNIN decomposing fungi, *Aspergillus clavatus*, *Penicillium martensii* and *Pythium proliferum* deBary were isolated from soil. The cultures were tested for their efficacy in decomposing wheat straw lignin in liquid medium as well as in soil. The major loss of lignin occurred within 4 weeks of inoculation and later the rate of decomposition was sluggish. *Aspergillus clavatus* was found to have comparatively higher lignoclastic activity.

Introduction

The decomposability of plant material in soil is largely determined by its nitrogen and lignin contents. Lignin, besides being resistant to decay, imparts a protective covering over easily decomposable plant constituents like celluloses and hemicelluloses. Among lignin decomposing organisms Basidiomycetes such as *Polystichus*, *Polyporus*¹ have been well known. Some fungi imperfectii like *Aspergillus*, *Penicillium* and ascomycetes also metabolize lignin^{2,4,7}. These heterotrophic microorganisms have great practical significance. The efficient strains of these microorganisms may be utilized to hasten the decomposition of crop residues in compost and manure pits.

Experimental

Lignin decomposing fungi were isolated from soil, compost and decaying wood on Czapeck's agar medium containing tannic acid. The colonies showing brown halo round them were considered as lignin decomposers (Fig. 1). The selected isolates *Aspergillus clavatus*, *Penicillium martensii* and *Pythium proliferum* deBary were tested for their efficiency in decomposing lignin in liquid medium containing (NH₄)₂ HPO₄ (0.1%), MgSO₄ (0.02%), KCl (0.002%) and also in soil. Wheat straw was uniformly cut to size (1 cm) and 4 g. samples were taken in 50 ml liquid medium in 250 ml conical flasks. In another series, 5 g wheat straw