CHAPTER I

INTRODUCTION
AND
AIMS OF THE STUDY
The occurrence of isatin (2,3-dioxoindole) as a natural metabolite in human and rat tissues was detected for the first time in 1988, although this compound has a long history. Isatin is one of the first organic compounds to be synthesized and its structure was characterized in 1841 as an indigo derivative (Glover et al., 1991). Isatin itself has a distinctive bright orange/yellow colour. It dissolves in hot water and also in organic solvents such as ethylacetate, although not in acetone.

Isatin has acquired immense importance due to its application in analytical biochemistry and many other fields of modern biology. It is well known as a pharmacological agent and its effects have been studied in a variety of systems (Glover et al., 1991). A review published by Varma and Khan (1978) provides a good deal of information on isatin and some of its derivatives as potential biologically active agents with antimicrobial, antineoplastic, anti-hypotensive, analgesic, anti-inflammatory and cysticidal properties in relation to its molecular structure. Some N-Mannich base derivatives of isatin appear to act as antiviral, antibacterial and antifungal agents (Joshi and Chand, 1982). Isatin itself also seems to selectively damage Echinococcus multilocularies, and may have a role in the chemotherapy of infections caused by this parasite (Delabre et al., 1989). It has also been reported that isatin,
produced by commensal bacterial attached to a certain shrimp, has a potent antifungal action which is necessary for the shrimp's survival (Gil-Turnes et al., 1989).

The CNS (Central Nervous System) affecting properties of isatin and its derivatives have been of considerable interest to researchers working for the development of newer drugs against epilepsy. Isatin has been shown to be endowed with the ability to cross blood-brain barrier and act as a potent and comparatively non-toxic anticonvulsant agent (Muller, 1962; Sareen et al., 1962). Popp et al. (1980) have reported that certain isatin derivatives exhibited better anticonvulsant potency than the parent compound. In rodents, isatin has been reported to act as an antiseizure agent in a variety of different tests (Sareen et al., 1962; Kohli et al., 1962; Muller, 1962; Chocholova and Kolinova, 1979; Popp and Pajouhesh, 1982; Pajouhesh et al., 1983). It has also been shown to reduce low wave sleep (Chocholova and Kolinova, 1981). The antiepileptic properties of isatin are attributed to its activated 3-keto group which can possibly bind with ammonia to prevent its excessive accumulation in brain.

Isatin has also been studied for its effects on membrane transport system. Nagpual et al. (1985) studied the inhibition of D-glucose uptake by isatin in rat intestine. Isatin inhibited both the sugar uptake and transmural
(mucosal to serosal side) transport in rat intestine. Similar to the action of various -SH group reacting agents, isatin inhibited the sugar uptake, presumably by binding to membrane sulfhydryl groups through a covalent linkage. Inhibition of sugar uptake by isatin and harmaline (a compound known to impair the transport process by specially interacting at ion binding site of transport carrier; Sepulveda and Robinson, 1974) was additive in nature, this suggested that these compounds interact at different sites on microvillus membrane surface.

Out of diverse biological responses isatins elicit, one is their ability to influence activities of several enzyme systems both in vivo and in vitro. The interaction of the functional groups present in the side chain of amino acids constituting the protein or enzyme with the 3-oxo groups of isatins have been of special interest to protein chemists and enzymologists.

Isatin inhibited the milk xanthine oxidase activity in a time and inhibitor concentration dependent manner (Bruns, 1954). In view of the fact that isatin reacts with amino acids by a Strecker type reaction, it has been concluded that the inhibition was the result of the reaction of the inhibitor with the enzyme protein, possibly resulting in deamination and decarboxylation of its N-terminal amino or carboxyl group.
Susheela et al. (1969) studied in vitro inhibition of rat liver xanthine oxidase by isatin. The inhibition was of non-competitive type and highly dependent upon concentration of inhibitor. They also reported that isatin administration orally increased the specific activity of rat liver and kidney xanthine oxidase. The different behaviour of isatin in vitro and in vivo might be due to its decomposition in the rat body. Isatin was shown to inhibit rat testicular alkaline phosphatase (Kumar et al., 1978). Singh et al. (1978) reported that the administration of isatin to rats diminished the activity of kidney alkaline phosphatase but enhanced the enzyme activity in duodenum and jejunum.

Isatin is a potent inhibitor of monoamine oxidase (MAO), particularly of MAO B and also binds to central benzodiazepine receptors. Muller (1962) reported that isatin inhibited MAO activity in mice liver homogenate. In a later communication, Muller and Schmiedel (1965) showed that administration of isatin, N-methyl isatin, dioxindole and oxindole inhibited MAO activity in mice liver homogenate. Isatin-B-hydrozones and isatin-B-imide exerted only an insignificant inhibitory effect. On the other hand, 5-Bromo isatin as well as 5-bromo isatin-B-hydrozone were found to be more potent inhibitors in comparison to isatin and isatin-B-hydrozone respectively. Very recently, Hamaue et al. (1992) reported the competitive inhibition of rat liver MAO activity by isatin.
Endogenous MAO inhibitory activity was first discovered in normal human urine by Glover et al. (1980) and was subsequently given the name "tribulin". In 1988, Glover et al. showed that direct probe analysis of a purified fraction has a mass spectrum identical to that of isatin. Isatin also had similar retention time as tribulin in all HPLC and TLC systems previously used in its purification (Elsworth et al., 1986). They concluded that the purified endogenous MAO inhibitor, "tribulin", corresponds to isatin and isatin is normally present in human urine and rat heart and brain. They further reported that the concentrations of isatin in human urine and rat heart and brain, are sufficient to account for MAO inhibitory activity previously reported by Armando et al. (1986) and Clow et al. (1988). Isatin has now been found to have quite distinct tissue distribution in various rat tissues and different regions of rat brain as described in Table 1 (Watkins et al., 1990).

Thus, it is amply evident from the literature that biological effects of isatins are diverse in different tissues. The most well characterized effects of isatin include, beside the others, (i) anticonvulsant agent, (ii) inhibition of MAO activity, and (iii) inhibitor of Na\textsuperscript{+} dependent sugar transport system in intestine. These systems although diverse in nature, have a common property of being affected by a monovalent cations. Therefore, it is reasonable to believe that this common property might be the underlying mechanism of isatin interaction with these systems.

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<table>
<thead>
<tr>
<th>Region</th>
<th>Concentration ug/g</th>
</tr>
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<tbody>
<tr>
<td>Whole brain</td>
<td>0.04±0.005</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>0.04±0.003</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>3.10±0.20</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>1.60±0.10</td>
</tr>
<tr>
<td>Heart</td>
<td>0.16±0.10</td>
</tr>
<tr>
<td>Liver</td>
<td>0.16±0.10</td>
</tr>
<tr>
<td>Testes</td>
<td>0.04±0.03</td>
</tr>
<tr>
<td>Urine</td>
<td>0.66±0.05</td>
</tr>
</tbody>
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Table 1: Distribution of isatin in rat brain and tissues
(Glover et al., 1991)
Brush border sucrase is another system which is well characterized and known to be influenced by Na⁺ ion and the metal ions interactions are pH dependent (Alvarado and Mahmood, 1979). The kinetics of Na⁺ stimulation of brush border sucrase and organic solute transport system in intestine also follows a close parallelism in various animal species investigated (Mahmood and Alvarado, 1975). The Na⁺ dependent uphill transport of the organic solutes is indirectly linked to Na⁺,K⁺-ATPase activity located at the basolateral site of the enterocytes.

Thus it would be of interest to study the effects of isatin and its derivatives on brush border sucrase and membrane bound Na⁺,K⁺-ATPase to illustrate the effect of metal ion binding sites on these enzyme systems.

The present studies were planned with the following objectives:

1. To study the effects of isatins [Isatin, 3-Hydroxy-3-acetonyl oxindole (HAO) and 3-Hydroxy-3-phenacyl oxindole (HPO)] on Na⁺,K⁺-ATPase of intestine, kidney and RBCs.
2. To study the effect of isatins on intestinal brush border sucrase activity in relation to metal ion stimulation of the enzyme.
3. To study the effect of isatin on Na⁺ dependent and independent amino acid transport system in intestine.
4. To study the interactions of isatin with sugar transport system in human erythrocytes. This system was selected to study isatin interactions, since glucose uptake in erythrocytes occurs by a facilitative transport mechanism and does not require any metal ions. Such studies will be useful to understand the mechanism of isatin interaction with transport system and to distinguish whether metal ions are required for isatin action.