Review

THYROID HORMONES REGULATE MITOCHONDRIAL RESPIRATION AS WELL AS ANTIOXIDANT DEFENSE IN TELEOSTS TOO!

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SUMMARY

Our studies describe the effect of T_3 on the regulation of mitochondrial metabolism, lipid peroxidation and antioxidant enzyme homeostasis in a fresh water teleost, *Anabas testudineus*. These experiments suggest the possibility that certain of the clinical and biochemical manifestations of T_3 can be attributed to a direct effect of T_3 on mitochondria. Thyroid hormone enhances mitochondrial ATP production rate in the highly oxidative tissue such as liver. These *in vitro* and *in vivo* studies substantiate and confirm the earlier reported T_3-induced oxidative metabolism in lower vertebrates. These induced oxidative metabolisms create oxidative stress in tissues. This confirms that T_3 has an immediate response in *in vivo* and *in vitro* system on energy adjustment in the fish, *A. testudineus.* Tri-iodothyronine is also capable of combating the oxidative stress by activating the antioxidant system to remove the ROS. Tri-iodothyronine appears to have a dual role, as a stimulator of oxidative process and as a regulator of antioxidant enzyme activity. This establishes another example for the multi-functional role of T_3 in lower vertebrates also. However, the precise mechanism of action remains to be understood. The protein expression study revealed that T_3 administration in fish creates hypermetaolic state. This hypermetabolic state creates oxidative stress in fish. To maintain the homeostasis of the fish, physiology the main antioxidant CuZn SOD is directly altered. Due to augmented SOD utilization, expression of CuZn SOD is diminished in fish liver and brain. These findings conclude that thyroid hormone effectively maintains physiological status of fresh water fish. It is obvious that thyroid hormones have an over all effect on metabolism in responsive tissue and that their effect is a direct one. Theses studies establish multi-functional role of T_3 in lower vertebrates.

Key words: *Anabas testudineus*, free radicals, mitochondrial respiration, superoxide dismutase, thyroid hormone, tri-iodothyronine

INTRODUCTION

The thyroid gland is genetically programmed to be the metabolic regulator in all vertebrates. As metabolic regulators, the thyroid hormones exert numerous effects on nearly all tissues of the body in every vertebrate so for investigated. They accelerate cellular reaction in most tissues of the body, with some exceptions, and are well known for their calorigenic effect in homeotherms. But in poikilotherms this effect of thyroid hormone is less studied. Thyroid hormones stimulate oxygen consumption in most of the tissues examined (1). At the biochemical level, the calorigenic action of thyroid hormones, as elicited by the regulation of basal metabolic rate, was considered to be of prime importance (2). For this reason, mitochondria have been considered as a site of hormone action at the level of the regulation of cellular respiration and energy metabolism.

Reactive oxygen species (ROS) are generated as by-products of normal tissue metabolism (3). It is also well established that hyperthyroidism in vertebrates leads to accelerated basal metabolic rate and oxygen consumption in several tissues (4). Accumulating evidence has suggested that the hypermetabolic state in hyperthyroidism is associated with increased free radical production and lipid peroxide levels (5). Moreover, it has been shown that tissues in hyperthyroid rats exhibit low antioxidant capacity and high susceptibility to oxidative challenge (6). A major biological process leading to ROS generation is electron transport within the inner mitochondrial membranes as a result of increased oxygen consumption (7). An imbalance between lipid peroxidation products and antioxidants leads to many biochemical changes which in turn can cause several chronic diseases in humans such as atherosclerosis, cardiovascular diseases, mutagenesis, cancer and several neurodegenerative diseases.

The past 10 years have seen tremendous progress in the definition of the nuclear mechanism of action of thyroid hormones. In teleosts, as in mammals, the primary thyroid hormones are tri-iodothyronine (T_3) and thyroxine (T_4) which have a wide range of effects on metabolic processes (8). However, the precise role of these hormones on oxidative metabolism in freshwater teleosts in relation to different enzyme systems remained to be understood (9).

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From a number of reports, it is understood that thyroid hormones stimulate mitochondrial respiratory functions in mammals (10). Kadenbach (11) had reported an increased basal metabolism of hyperthyroid tissues. It has long been known that an increase in metabolic rate follows administration of a sufficient dose of thyroid hormone. Thyroid hormones manifest an increased rate of oxygen consumption, a rise in body temperature, and a loss in weight, all consequent to an increase in the rate of oxidation of substrate (12). These studies have shown, for example, that thyroid hormones stimulate the rate of mitochondrial oxidation of succinate, glutamate, beta-hyroxybutyrate and isocitrate (13) and the activity of the membrane-bound alpha - glycerophosphate dehyrogenase (14). In addition, thyroid hormone affects mitochondrial swelling (15) and respiratory control ratio (16). But these effects were observed with large doses. Last 25 years of research in our laboratory established a definite role for thyroid hormone on oxidative metabolism in lower vertebrates. In view of these reports and lack of adequate information in fishes, studies were performed to elucidate the effect of T_3 on the respiratory rate of isolated liver mitochondria of a teleost, *Anabas testudineus* (Bloch).

Although molecular oxygen, which is an essential element in aerobic metabolism, is relatively unreactive, it is a potential source of reactive forms, such as free radicals. These species are highly reactive and often destructive to other molecules in the vicinity of their production. Under normal physiological conditions, there appears to be four key sources for the generation of free radicals. They are mitochondrial electron transport, peroxisomal fatty acid metabolism, cytochrome P-^{450} reactions and the respiratory burst (17). In aerobic cells, oxygen radicals and related free radicals are generated during normal metabolism in the mitochondria. The tetravalent electron reduction of molecular oxygen to water is one of the important reactions that take place in mitochondrial electron transport chain (ETC) during cellular respiration, which is essential for the existence of living organisms (18). The full reduction of oxygen to H_2O by cytochrome oxidase is a key step in the mechanism of aerobic ATP formation. Transfer of electrons between oxygen species allows each of us to survive on this planet, not only at the cellular level but also as an organism. The ETC, which is found in the inner mitochondrial membrane, utilizes oxygen to generate energy in the form of adenosine triphosphate (ATP). Oxygen acts as the terminal electron acceptor within the ETC. The literature suggests that anywhere from 2 to 5% of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron escape (19).

The important and effective defense mechanism against ROS is SOD. Superoxide dismutase (SOD) is a metalloenzyme whose active center is occupied by copper and zinc, sometimes manganese or iron. SOD plays an extremely important role in the protection of all aerobic life-systems. The SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. The SOD is an endogenously produced intracellular enzyme present in essentially every cell in the body. There are at least three forms of SOD in nature. Human erythrocytes contain SOD with divalent copper and divalent zinc. Chicken liver mitochondria and *E. coli* contain a form with trivalent manganese. *E. coli* also contains a form of the enzyme with trivalent iron. The two subunits are joined by a disulfide bond. The SOD plays major roles in the protection of cells against oxidative damage. The two major forms of SOD in humans are the mitochondrial manganese SOD and the cytosolic copper/zinc SOD. The Mn SOD protects mitochondrial proteins, membranes and DNA from O_2^- generated as a result of the respiratory activity. A CuZn-SOD has been used intra-articularly for degenerative joint disorders as an anti-inflammatory agent. SOD is also marketed as a nutritional supplement. The most deleterious ROS are hydroxy and superoxide radicals. To protect from this threat, cells have SOD and catalase enzymes. Hydrogen peroxide is itself deleterious and is destroyed by catalase. Catalase makes the conversion of H_2O_2 into water and oxygen. The Glutathione redox cycle is another mechanism which scavenges H_2O_2. Glutathione peroxidase reduces H_2O_2 to H_2O by oxidizing glutathione. Reduction of the oxidized form of glutathione is then catalysed by glutathione reductase. The glutathione cycle is complementary to catalase in scavenging H_2O_2. The present review describes a short-term effect of T_3 on antioxidant enzyme activities in a teleost, *A. testudineus.* Oxygen consumption by mitochondria is greatly influenced by their respiratory state (20). There may be greater chance of regulation of lipid peroxidation by these hormones. Further, it was reported that lipid peroxidation and antioxidant enzyme activities are regulated by T_3 (21). However, our study made it clear that there is an over all dependence of thyroid status on lipid peroxidation and antioxidant enzyme activities in lower vertebrate like *A. testudineus.* Thyroid hormones are known to influence cellular oxygen consumption, oxidative phosphoryation and proton leak (22). The oxygen consumption by mitochondria is generally influenced by their respiratory state (20, 23). However, short-term effect of thyroid hormones on antioxidant enzymes remained to be investigated.

ANABAS TESTUDINEUS AS A MODEL ORGANISM

The rationale for the selection of the Indian perch *A. testudineu*s as a model animal is that its thyroid structure and function resemble other vertebrates. Besides, a strong antioxidant defense system is reported in fish (24). In an earlier study from our laboratory, we established a delayed effect by T_3 on the regulation of antioxidant enzyme activities of *A. testudineus* (25). In our several studies subsequently, we have examined the short-term *in vivo* and *in vitro* action of T_3 on *A. testudineus* to confirm whether thyroid hormones also protect the deleterious effect of ROS generated during increased oxidative metabolism. (Table 1, 2).

Table 1

Effect of T_3 on state 4 & state 3 respiration in the liver of 6-PTU treated *Anabas testudineus**- an *in vivo* and *in vitro* study

	In vivo				*In vitro*				
	Control	**C-PTU**	**6-PTU+T_3 (30min)**	**6-PUT+T_3 (60 min)**	**Control**	**6-PTU**	**6-PTU+T_3 (10 min)**	**6-PTU+T_3 (15 min)**	**(6-PTU+T^3 (30 min)**
State.4	1.54±0.08[a]	0.52±0.06[b]	1.35±0.15[c]	0.70±0.06[b]	1.65±0.13[a]	0.33±0.06[b]	1.36±0.31[a]	2.05±0.22[a]	3.92±0.37c
State.3	0.98±0.24[a]	0.34±0.08[b]	0.55±0.04[c]	0.41±0.04[b]	0.55±0.09[a]	0.27±0.02[b]	0.71±0.26[a]	3.20±0.29[a]	2.71±0.22c

Table 2

	In vivo			*In vitro*			
	Control	**T_3 alone (30min)**	**T_3 alone (60min)**	**Control**	**T_3 alone (10 min)**	**T_3 alone (10 min)**	**T_3 alone (30 min)**
State.4	1.54±0.08[a]	0.55±0.10[b]	2.00±0.18[c]	1.65±0.13[a]	2.72±0.33[b]	2.72±0.62[b]	2.00±0.14[ab]
State.3	0.98±0.24[a]	0.27±0.04[b]	1.38±0.34[c]	0.55±0.09[a]	2.34±0.07[b]	1.60±0.31[b]	1.41±0.10[c]

*Results are expressed as mean ± SE of 8 animals (n=8). The significant difference between the groups was analyzed by one way analysis of variance. IU for state 4 and state 3 respiration; oxygen concentration nmol/min Mean values of different superscript alphabets (a, b, c) are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

STIMULATORY RESPONSE OF RESPIRATION TO T_3 TREATMENT

A short-term *in vivo* and *in vitro* stimulatory response of state 3 and state 4 respiration to T_3 treatment is clearly demonstrated in our studies. T_3 stimulates both the production and consumption of energy within the cells. Probably, thyroid hormone can be recognized as a major regulator of oxidative energy metabolism at the level of the mitochondria in teleost fish too. Mitochondria isolated from thyroid hormone-treated animals display increased àO_2 rates (26) due to accelerated import and oxidation of fuel substrate (27). This increased metabolic flux is also facilitated by higher activities of enzymes in the oxidative pathway (28). An important aspect of mitochondrial metabolism yet to be fully defined in the hyperthyroid state is ATP production. The increase in substrate oxidation (29) and enzymatic capacity (30) in various tissues suggested that ATP production capacity may be enhanced by thyroid hormone. It is suggested that hyperthyroidism leads to a greater increase in capacity to ATP production in short term period. It clearly reveals that increased metabolic rate and hyperactivity are well documented in hyperthyroid condition. The most widely accepted idea is that held by those scientists who believed that the effects exerted by T_3 on mitochondrial activities are indirect, all of them being mediated by an early effect at the nuclear level (31).

An analysis of the sequential biochemical changes induced by thyroid hormone reveals that nuclear RNA synthesis preceded biochemical changes in mitochondria. These findings raised the possibility that nuclear events mediate thyroid hormone-stimulated mitochondrial metabolism. This hypothesis indicated that thyroid hormones exert multiple independent effects via specific receptors located in the nuclei of target tissues. Mechanisms of the effects of thyroid hormone on oxidative processes have focused considerable attention on the mitochondria as a potential direct target for hormone action (32). Mitochondrial binding site T_3 may play a very important physiological role in regulating the mitochondrial transcription apparatus. This is reasonable for two reasons: (a) T_3 influences the mitochondrial biogenesis and turnover,

(b) the mitochondrial biogenesis or turnover needs the co-ordinated participation of the nuclear and mitochondrial genetic apparatus. In fact, the early results obtained from our laboratory and by others show that T_3 regulates the mitochondrial population and the mitochondrial nucleic acid level (33). Sterling *et al.* (34) reported the existence of specific mitochondrial binding sites for thyroid hormone.

MITOCHONDRIA AS TARGETS FOR EARLY ACTION OF T_3

In the past, investigators frequently assumed that the calorigenic effects of thyroid hormone reflected a simple effect on energy production. Because thyroid hormones regulate oxygen consumption, once the role of mitochondria in this process was established, they appeared to be likely targets for hormone action. Direct action of T_3 on mitochondria has been suggested based on three pieces of evidence. First, high-affinity binding sites were located in the mitochondrial membrane (35). However, as discussed above, evidence linking these sites to the biological function of the hormone is still lacking. Second, some effects of T_3 on mitochondria are so rapid that it is difficult to envisage a nuclear pathway operating in that span of time (36). Third, thyroid hormone may have direct effects on isolated mitochondria (37). Although adding thyroid hormone to isolated mitochondria *in vitro* simulated the amino acid incorporation, different mechanisms appear to initiate these processes (38). Regardless of whether T_3 has direct non-nuclear actions on mitochondria, at least some of the calorigenic effects of the hormone are manifested by changes in these organelles. Thyroid hormone has a general stimulatory effect on mitochondrial biogenesis (39), particularly on the surface area of the inner membrane (40).

T_3 AND ANTI-OXIDANT DEFENCE SYSTEM

A rapid *in vivo* and *in vitro* regulatory effect of T_3 on the antioxidant defense system in a teleost *A. testudineus* is reported in this study. SOD was high in 6-PTU and 6-PTU plus thyroid hormone treated fish (Table 3, 4, 5). This clearly reveals that 6-PTU produces reactive oxygen species, which in turn enhances defense mechanism through the activation of enzymes. 6-PTU treatment elevated SOD activity in liver mitochondrial fraction and also catalase activity (41). The results on lipid peroxidation in liver homogenate also corroborate the previous studies, where T_3 treatment resulted in a significant increase of lipid peroxidation in rat liver (42). The reason is that increased free radical production decreased antioxidant activities. Due to this reason hormone administration inhibits the activity of SOD and catalase in the liver (41). The present results suggest that the antioxidant defense status of liver is well modulated by thyroid hormone in *Anabas*. The administration of T_3 in normal fish increases the activity of SOD and catalase in *in vivo* and *in vitro* study. This is indicative of a short-term time-dependent effect of thyroid hormone on cellular defense mechanism in the teleost. Hyperthyroidism is a hypermetabolic state accompanied by increased oxygen utilization, increased production of ROS and, consequently, measurable changes in antioxidative factors and increased SOD activity as reported by Mayer *et al.* (43). The increased production of H_2O_2 as a result of the removal of reactive oxygen species by SOD may stimulated the catalase activity. Hydrogen peroxide is formed in cellular systems due to dismutation of superoxide radicals by the enzyme SOD and reduction of H_2O_2 is catalyzed by catalase and GPx (44). The formation of superoxide, activated by thyroid hormone, may occur mainly in the mitochondrial respiratory system.

Thyroid-hormone status has many effects on the expression of hepatic enzyme systems. Hyperthyroidism resulted in a marked increase in intracellular antioxidant enzymes *i.e.*, catalase and GPx activities as compared to the control (45). Hypothyroidism induced by 6-PTU is accompanied by an increase in hepatic GPx, which has been attributed to an increase in the GPx and can be reversed by intraperitoneal injection of T_3. This is also in the case of glutathione transferase because these two hepatic enzymes are interlinked (46). Stimulation of antioxidant in liver following hypothyroidism and subsequent inhibition of its level by T_3 administration confirm important role of thyroid hormone in controlling oxidative stress state and prevention of free radicals in liver in this study. In hypothyroidism, liver SOD, catalase and GPx activities were elevated and reversed by T_3 confirming that thyroid hormone may be responsible for regulating H_2O_2 level in liver. The level of H_2O_2 in the liver is closely associated with the activities of SOD, catalase and GPx. The surprising evidence is that increased liver H_2O_2 level in hypothyroid fish liver is not associated with an elevation of lipid peroxidation products probably because glutathione system in liver tissue is a major cellular antioxidant (47). We produced evidence to suggest strongly that H_2O_2 content in liver is under in thyroid hormone regulation. Decrease in the level of thyroid hormone invariably elevates SOD, catalase and GPx activities, and T_3 inhibits them giving strong evidence of direct control of thyroid hormone on defense mechanism. Hyperthyroidism is a hypermetabolic state accompanied by increased oxygen utilization, increased production of reactive oxygen species and consequently measurable changes in antioxidative factors. The rapid *in vivo* action of T_3 on antioxidant enzyme

activities is substantiated by similar *in vitro* results in *A. testudineus*. In hyperthyroid rat liver, besides higher lipid peroxidation, a more active defense mechanism operates as in euthyroid rats (48). T_3 is capable of combating the oxidative stress by activating the antioxidant enzyme systems by removing the ROS. T_3 appears have a dual role, as a stimulator of oxidative process and as a regulator of antioxidant enzyme activity (49). Thus, it was revealed that there exists a delicate balance between the rate of formation and breakdown of ROS in the liver, which is under the subtle control of thyroid hormone. Any alteration in thyroid activity probably results in a transient physiological stress, which would influence lipid peroxidation and antioxidant enzyme activities. This means that an internal homeostatic balance between lipid peroxidation and antioxidant enzymes is dependent on the thyroid status (Table 3, 4, 5).

Table 3: Effect of T_3 on lipid peroxidation & antioxidant enzyme activity in the liver of 6-PTU treated teleost A. *testudineus, an in *vivo* study**

Parameter studied	Control	6-PTU	6-PTU+T3 (15 min, 200 ng)	6-PTU+T3 (30 min, 200 ng)	6-PTU+T3 (60 min, 200 ng)
Catalase (IU/mg protein)	0.25± 0.02[a]	4.06± 0.35[b]	1.66± 0.21[c]	0.17± 0.006[a]	0.46± 0.06[a]
SOD (Units/mg protein)	5.90± 0.72[a]	15.38± 1.30[b]	8.76± 0.92[a]	7.02± 0.34[a]	11.80± 1.32[b]
Glutathione reductase (IU/mg protein)	4.46± 0.25[a]	6.77± 0.45[a]	4.57± 0.56[a]	3.99± 0.18[a]	16.71 4.51[b]
Glutathione peroxidase (IU/mg protein)	0.48± 0.02[a]	1.74± 0.27[b]	1.53± 0.40[b]	0.65± 0.02[a]	1.40 0.08[b]
Glutathione content (mmol/ 100g tissue)	2.61± 0.09[a]	2.58± 0.22[a]	2.52± 0.14[a]	1.76± 0.14[b]	3.59 0.08[c]

The SDS-PAGE and Western blot analysis revealed that T_3 treatment decreases protein expression of CuZn-SOD (Fig. 1). The significant reduction of CuZn-SOD in the liver after the T_3 treatment is due to the oxidative stress condition in these tissues. Tri-iodothyronine treatment in normal fish creates a hyperthyroid condition. This hyperthyroid condition creates hyper-metabolic state and generates lot of ROS resulting in oxidative stress. To contain the oxidative stress, antioxidants like CuZn-SOD acts immediately on ROS. The SOD activity increases ultimately resulting in decreased protein expression in liver when analyzed.

Table 4: Effect of T_3 on lipid peroxidation and antioxidant enzyme activity in the liver of normal *A. testudineus - an in *vivo* study**

Parameter studied	Control	T_3(15 min) (200 ng)	T_3(30min) (200 ng)
Catalas (IU/mg protein)	0.25±0.02[a]	0.79±0.12[b]	2.47±0.18[c]
SOD (units/mg protein)	5.90±0.72[a]	5.60±0.27[a]	10.18±0.41[b]
Glutathione (reductase IU/mg protein)	11.26±2.24[a]	11.20±1.60[a]	7.28±0.26[a]
Glutathione (peroxidase IU/mg protein)	4.80±0.20[a]	16.30±0.80[b]	17.80±2.50[b]
Glutathione (content mmol/ 100 g tissue)	2.60±0.09[a]	1.86±0.09[b]	2.72±0.08[a]

Table 5: Effect of T_3 on antioxidant enzyme activity in the liver of normal *A. testudineus** - an in *vivo* study

Parameter studied	Control	T_3(15 min) (10^{-6} *M*)	T_3(30min) (10^{-6} *M*)
Catalas (IU/mg protein)	0.55±0.023[a]	0.81±0.040[b]	1.08±0.14[c]
SOD (units/mg protein)	5.30±0.12[a]	6.10±0.23[a]	6.02±0.52[a]
Glutathione (reductase IU/mg protein)	11.70±1.30[a]	12.24±0.90[a]	18.45±1.38[b]
Glutathione (peroxidase IU/mg protein)	6.22±0.26[a]	10.99±0.69[b]	8.09±0.84[a]
Glutathione (content mmol/ 100 g tissue)	1.60±0.06[a]	1.50±0.08[a]	1.88±0.10[b]

*Results expressed as mean ± SE of 8 animals (n=8). The significant difference between the groups was analyzed by one-way analysis of variance. IU for GPx and GR-nmoles NADPH oxidized /min/mg protein and catalase was nmoles

H_2O_2/min/mg protein Mean values of different superscript letters (a, b, c) are significantly different ($P<0.05$) as determined by Duncan's multiple range test.

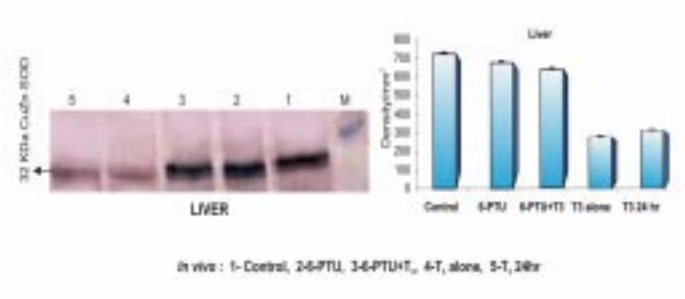

Fig.1. Western blot analysis of CuZn-SOD

POSSIBLE MECHANISM OF ACTION OF THYROID HORMONE ON LIPID PEROXIDATION AND ANTI-OXIDANT ENZYMES

Thyroid hormone action is mediated by multiple thyroid hormone receptor isoforms derived from two distinct genes. The thyroid hormone receptors belong to a nuclear receptor super-family that also includes receptors for other small lipophilic hormones. They function by binding to specific thyroid hormone-responsive sequences in promoters of target genes and thereby regulate transcription (50). Thyroid hormone may affect transport of solutes across membrane and the metabolism of such of those metabolites that are the principal sources of energy. They may initiate these effects directly or indirectly by generating a second messenger or modifying genetic expression particularly in the transcriptional and translational phasee of protein synthesis. The mechanisms of action of various hormones have challenged the imagination of biologists for over half a century and spurred numerous speculations and suggestions. The theory that the thyroid hormone may act directly on the transcriptional and translational processes involved in protein biosynthesis has considerable experimental support. The flow of information that determines the kind of protein synthesized is known to involve three processes *viz*, replication, transcription and translation (51). Thyroid hormone action is initiated through interaction with a nuclear receptor, and the subsequent alteration of cellular RNA production is certainly a plausible one. In this context, central role of T_3 in mediating thyroid hormone action and the recognition of specific nuclear receptors in target tissue is demonstrated by displacement studies (52). The rapid and specific stimulation of messenger RNA production for certain thyroid responsive proteins strengthen the concept of a direct action of hormone on gene expression. The relationship between the nucleic acid and protein biosynthesis to establish the site of thyroid hormone actions is concentrated on transcription and translational systems, involving mRNA and ribosomes, the protein synthesis units of the cell. To test the dependence of biological phenomenon on some aspects of protein biosynthesis is to determine whether they are sensitive to protein synthesis inhibitors, usually actinomycin-D or puromycin. In addition to the well-established nuclear action of T_3, effects of thyroid hormone on other sites including cell membranes and mitochondria have been documented (53). It has emerged in the last decade that the molecular mechanism of action of thyroid hormones resembles that of the steroids. They exert their effects mainly by directly regulating gene expression, on association with specific chromatin-bound receptors. Many cellular compartments other than nucleus bind thyroid hormone (54). There is plenty of information on the mechanism of thyroid hormones' action in mammalian system. However, there is very little information on this in lower vertebrates, especially fishes.

Lipid peroxidation product malondialdehyde increases in T_3 treated groups in *in vivo* and *in vitro* study and act-D treatment prevented the T_3 effect. The lipid peroxidation products decreased in act-D + T_3 treated fish in *in vitro* study. The increase in lipid peroxidation products after T_3 treatment may be due to hypermetabolism. Acceleration of basal metabolic rate and the energy metabolism of tissue in animal species represent one of the major functions of thyroid hormones (55). Several evidences have suggested that the hypermetabolic state in hyperthyroidism is associated with increases in free radical production and lipid peroxide levels (21). The significant increase of lipid peroxidation was reported by Fernandez *et al*. (56) in the liver of hyperthyroid rats. The studies have suggested that increase in ROS induced by thyroid hormone leads to an oxidative stress condition in liver (57) with a consequent lipid peroxidative response (Fig. 2).

ACTINOMYCIN-D INHIBITS THYROID HORMONE ACTIVITY

The patho-physiological consequences of the accelerated lipid peroxidation were elucidated. The biochemical changes are thought to be responsible for some complications of hyperthyroidism. Recently, it has been suggested that hyperthyroid state-induced biochemical changes predispose in liver (58) for free radical-mediated injury. Das and Chainy (42) also reported that T_3 treatment for three days resulted in a significant increase of lipid peroxidation in liver. These observations clearly reveal that T_3 creates hyper metabolic state in tissues. This hyper-metabolism creates free radical production. This is the

reason for increased free radical resulting in increased lipid peroxidation. Act-D, a potent transcription inhibitor is used to assess whether the specific treatment alters the stability of mRNA. The act-D binds tightly and specifically to double helical DNA and thereby prevents it from being an effective template for RNA synthesis. Thomas *et al*. (59)

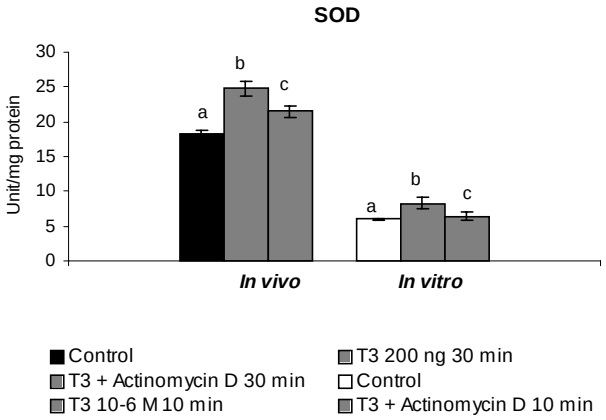

Fig. 2A.i. Effect of T_3 on SOD in the liver of act-D treated *Anabas testudineus.* *[Each histogram represents mean ± SE of 6 animals. Groups with different alphabet headings are significantly different (P<0.05)].

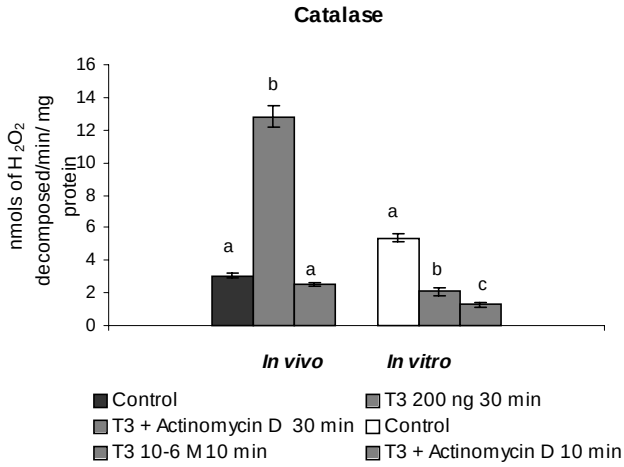

Fig. 2A.ii. Effect of T_3 on catalase in the liver of act-D treated *A. testudineus.* (*See foot note, Fig. 2A.i. for details).

reported that the number of T_3 binding sites after a T_3 injection appeared to be completely prevented by act-D, inhibitors of protein synthesis and RNA synthesis. Act-D prevents the T_3-induced lipid peroxidation in *Anabas*. That can be the reason for decrease in peroxidation products. Act-D inhibits protein synthesis and correspondingly prevents hypermetabolism. These studies confirm that act-D inhibits thyroid hormone activity resulting in reduced metabolic rate and free radical production. Saumya et al. (60) study also suggest that antioxidant defence status in *A. testudineus* is modulated by thyroid hormone, through an action sensitive to actinomycin-D

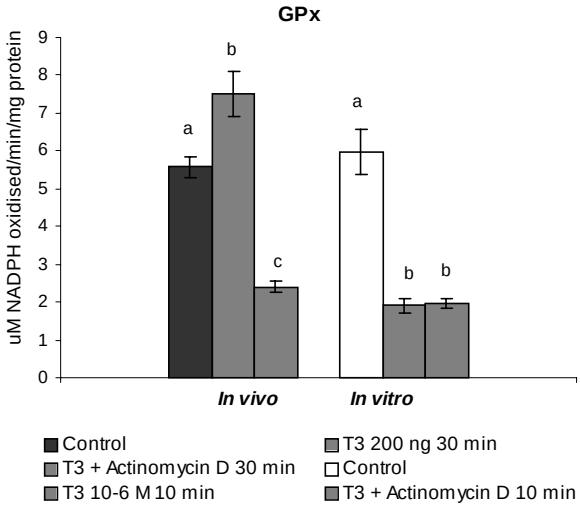

Fig. 2A.iii. Effect of T_3 on GPx in the liver of act-D treated *A. testudineus.* (*See foot note, Fig. 2A.i. for details).

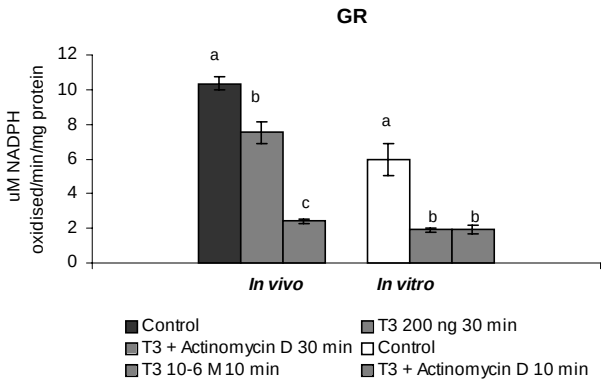

Fig. 2A.iv. Effect of T_3 on GR in the liver of act-D treated *A. testudineus.* (*See foot note, Fig. 2A.i.).

SUMMARY AND CONCLUSIONS

The increased understanding of the structure and function of thyroid hormone receptors and their interacting proteins has markedly clarified the molecular mechanisms of thyroid hormone action. Most of the effects of thyroid hormones are now known to occur through the actions in nuclear receptors that cause alteration in gene expression. Many of the effects of thyroid hormones in other tissues can be related to the action demonstrated herein, and this observation may be suggested as a general hypothesis for the mechanism of action. There is very little idea about mechanism of action thyroid hormone in lower vertebrates so far. Thus this work is a basis for future research on

thyroid hormone. Further studies are necessary to test this hypothesis and also to define the nature of the interaction of the thyroid hormones and their receptors to regulate antioxidant and lipid peroxidation products.

ACKNOWLEDGMENT

The authors thank the Department of Science and Technology (DST), Govt. of India (SP/S0/C12/2000), and DST- FIST & KSCSTE-SARD programmes for financial assistance.

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