Free Rad. Res., Vol. 21, No. 5, pp. 285-294
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DEVELOPMENTAL ASPECTS OF DETOXIFYING ENZYMES IN FISH (*SALMO IRIDAEUS*)

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(Received March 26th, 1994; in revised form May 4th, 1994)

The activities of superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione transferase and glyoxalase I have been studied during the embryologic development of rainbow trout (*Salmo iridaeus*) and in several other trout tissues to investigate the protective development metabolism.

A gradual increase of superoxide dismutase, catalase, glutathione reductase, glyoxalase I and glutathione transferase activities was noted throughout embryo development.

In all trout tissues investigated glutathione peroxidase was found to be extremely low compared to catalase activity. The highest activity of superoxide dismutase, glyoxalase I and glutathione reductase was found in liver followed by kidney.

No change in the number of GST subunits was noted with the transition from the embryonic to the adult stages of life according to the SDS/PAGE and HPLC analyses performed on the GSH-affinity purified fractions.

KEY WORDS: Superoxide dismutase, catalase, glutathione reductase, glyoxalase I, glutathione transferase, development.

INTRODUCTION

The utilisation of oxygen by a variety of biochemical reactions produce partially reduced oxygen species, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). These metabolites are cytotoxic, being able to produce deleterious effect on vital cellular macromolecules.[1] The removal of damaging oxygen products is catalyzed by antioxidant enzymes including superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GSH-Px; 1.11.1.9). SOD removes the superoxide anion in a dismutation reaction producing hydrogen peroxide and oxygen. The removal of hydrogen peroxide thus formed can be catalyzed by either CAT or GSH-Px.[2] At least two different GSH-Px activities are identified in animal tissues.[3] The former, in addition to hydrogen peroxide, is able to reduce any other organic hydroperoxides (selenium-dependent GSH-Px); the latter one which can reduce only the organic hydroperoxides (selenium independent GSH-Px), has been demonstrated to arise

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from particular isoenzymes of glutathione transferase[4] (GST; EC. 2.5.1.18). In the peroxidase reaction GSH is oxidized to GSSG. The regeneration of GSH is consequently of fundamental importance for the ability of cells to withstand exposure to oxidizing metabolites. In the cell, GSSG is enzymatically reduced by the action of glutathione reductase (GSSG-Rx; 1.6.4.27) in the presence of NADPH. GST are a family of multifunctional enzymes that, in addition to the reduction of organic hydroperoxides, catalyse the conjugation to GSH of a large variety of electrophilic alkylating compounds, including hydroxyalkenals, thereby protecting cells against their potential toxicity.[5] On the other hand, certain reactions involving endobiotics as well as xenobiotics may lead to the formation of 2-oxoaldehydes that may be eliminated through the action of a glyoxalase system[6,7] (GLXI 4.4.1.5).

A great number of experimental studies have been published on the alteration occurring in these detoxifying enzymes in hepatic and extrahepatic tissues of different animals, including man, during foetal and postnatal development.[8-13] On the contrary, little is known about the level of these enzymes during embryo development and their possible changes occurring in the transition from embryonic to adult life.[14-18] In Amphibian, (*Bufo bufo*), an increase of GSH-PX, CAT and GST activity was observed after fertilization, and, while GST activity decreases during development CAT rises in a fashion coherent with respiration.[15-17] GSSG-Rx activity, after a slight fall from stage 4 to stage 7, constantly increases up to the end of development[15]. In addition, in Amphibia, with the passage from the embryonic to the adult life a change in the GST isoenzyme expression also occurs.[18-20] In particular, a GST isoenzyme which is able to reduce organic hydroperoxides not expressed during embryonic life, is present in toad liver and kidney.[18-20]

In the present investigation the developmental pattern of CAT, SOD, GSH-Px, GLX I, GSSG-Rx and GST was studied in fishes (*Salmo Iridaeus*), animal species which do not change their aquatic environment during the transition from embryonic to adult life.

MATERIALS AND METHODS

Sample

Salmo iridaeus embryos as well as young and adult rainbow trout (*Salmo iridaeus*) were supplied from dr. Maurizio Crispan (SIM, Popoli, Italy). The embryos were staged by reference to Oppenheimer.[21]

Cytosol Preparation

After three washings with 0.01M potassium phosphate buffer pH 7.0 the embryos (8–16 embryo/ml) and the trout tissues were suspended (1:5 w/v) in the same buffer made 1% with respect triton X100 (for CAT and SOD activity) or made 1 mM dithiothreitol (for GST, GSSG-Rx, GLXI and GSH-Px activities) and homogenized with a Potter homogenizer by hand with ten pestle strokes. The homogenate was centrifuged for 60 min at 105,000 xg with a Spinco L-50 centrifuge. The supernatant was recovered by a syringe and used for the enzymatic activity measurements and for GST purification.

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GST Purification

The 105,000 xg supernatant was applied to a GSH-Sepharose affinity column[22] that had been pre-equilibrated with 10 mM potassium phosphate buffer pH 7.0. The column was first washed with the equilibrating buffer, supplemented with 200 mM KC1 and then eluted with 50 mM Tris/HC1 buffer, pH 9.6, containing 20 mM GSH. The fractions containing GST activity were pooled, concentrated by ultrafiltration, dialysed against 10 mM potassium phosphate buffer pH 7.0, supplemented with 1 mM dithiothreitol and used for further characterization.

SDS/PAGE

SDS/PAGE was performed as described by Laemmli.[23] SDS concentration was 0.1%, the spacer gel and the separating gel were 3% and 12.5% acrylamide, respectively. Phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovoalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) were used as standards proteins. Western blots are made essentially according to the protocol described by Towbin *et al.*[24]

Separation Of GST Subunits By H.P.L.C.

H.p.l.c. analysis was performed by using the method described by Ostlund-Farrants *et al.*[25] A Waters μBondapak C_{18} (0.39 × 30.0 cm) column attached to a Kontron h.p.l.c. system was used. The column was developed at 1 ml/min by a 45 min gradient from 35% to 55% acetonitrile in 0.1% (v/v) triflouroacetic acid; this was followed by a 55–70% acetonitrile gradient in 0.1% trifluoracetic acid formed over 2 min. The eluate was monitored at 220 nm.

Enzymatic Assay

Catalase

CAT activity was measured by using a YSI oxygraph according to Del Rio *et al.*[26] The reaction mixture consisted of 3 ml of 67 mM sodium-potassium phosphate buffer pH 7.0 and 80 mM H_2O_2. The reaction mixture was deoxygenated by bubbling pure nitrogen, and the reaction was started by adding pure cytosol. The temperature was 25 °C. The readings monitored on a recorder were corrected for the spontaneous H_2O_2 decomposition. CAT units were defined as μg of O_2 produced/min.

Superoxide dismutase

SOD activity was determined by the epinephrine method as described by Sun and Zigman.[27] The inhibitory effect of SOD on the autoxidation of epinephrine (0.1 mM) in 50 mM sodium carbonate buffer, pH 10.0, was assayed spectrophotometrically at 320 nm and 25 °C. Percent inhibition values were converted into activities by using a purified Cu/Zn bovine SOD standard. One unit of SOD was defined as the amount of the enzyme required to halve the rate of substrate autoxidation.

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Glutathione peroxidase

Quantification of GSH-Px activity was done by the method of Paglia and Valentine[28] as modified by Di Ilio *et al.*[29] The activity of the Se- independent GSH-Px was determined by measuring total glutathione peroxidase activity with cumene hydroperoxide (1.2 mM) as substrate, and then subtracting from this activity the Se-dependent GSH-Px activity measured with H_2O_2 (0.25 mM) as substrate. The oxidation of NADPH was followed at 25 °C with a Kontron spectrophotometer. One unit was defined as 1 μmol of GSH oxidized/min.

Glutathione reductase

GSSG-Rx activity was measured as described previously.[30] The assay mixture contained 0.1 mM potassium phosphate buffer pH 7.4, 1 mM EDTA, 1 mM GSSG and 0.16 mM NADPH. The blank did not contained GSSG. The oxidation of NADPH was followed at 25 °C with a Kontron spectrophotometer. One unit was defined as 1 μmol of NADPH oxidized/min.

Glutathione transferase

GST activity was recorded at 340 nm and at 25 °C by the method described by Habig and Jakoby.[31] The standard assay mixture contained 0.1 M potassium phosphate buffer pH 6.5, 1 mM EDTA, 2 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene. The conjugation reaction was monitored at 25 °C with a Kontron spectrophotometer following the increase of absorbance at 340 nm. One unit of enzyme activity was defined as 1 μmol of GSH conjugated/min.

Glyoxalase I

GLX I activity was monitored as described by Mannervik *et al.*[32] The assay solution contained 0.1 M sodium potassium buffer pH 7.2, 2 mM methylglyoxal and 1 mM GSH. The reaction was monitored at 25 °C, with a Kontron spectrophotometer, following the increase of absorbance at 240 nm. One unit of enzyme activity was defined as 1μmol of S-lactoylglutathione produced/min.

Protein

Protein concentration was determined with the method of Bradford[33] using γ-globulin as standard.

RESULTS AND DISCUSSION

The activity profiles of SOD, CAT, GSH-Px, GSSG-RX, GLX I and GST during fish (*Salmo iridaeus*) embryo development are presented in Figure 1. GSH-Px activity as measured with both H_2O_2 or cumene hydroperoxide was found to be undetectable in our experimental condition up to the end of embryo development when, a very low level of activity was measured. On the other hand CAT activity exists abundantly in *Salmo iridaeus* embryo. Thus, among the H_2O_2-removing systems CAT seems to be the only enzyme present, at significant level, in *Salmo*

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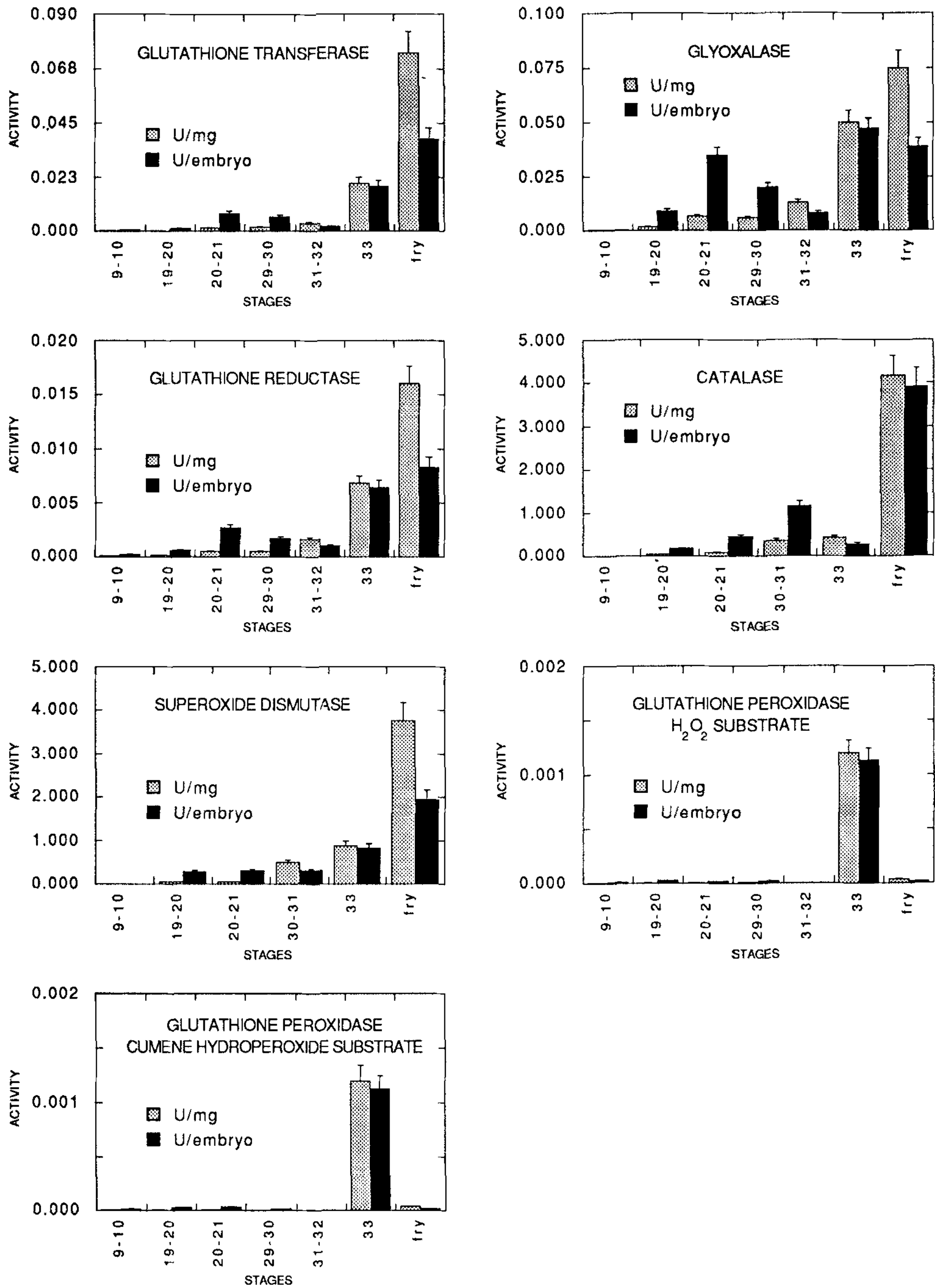

FIGURE 1 Glutathione transferase, glyoxalase I, glutathione reductase, catalase, superoxide dismutase and glutathione peroxidases activities during fish (*Salmo iridaeus*) development. Values plotted are means of quadruplicate assay of the same cytosol. SE never exceeded 5% of the mean.

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iridaeus embryo. On the basis of this result, it is reasonable to assign a more important role to CAT relative to GSH-Px in preventing H_2O_2 damage in fish embryo. With the progress of development a constant increase of SOD, CAT, GSSG-Rx, GLX I and GST activity was observed. For all enzymes maximum specific activity was measured at stage 33 when the formation of the swim bladder as well as the pigmentation has occurred. At the end of development GSSG-Rx, GST and GLXI activity values were found to be about 85-111- and 312-fold higher than those measured at the early stages. The variations of SOD and CAT from the early to the late stages of development were lower but substantially identical. At the fry stage, a further increase of activities occurs.

In a previous work CAT, GSH-Px, GSSG-Rx and GST were studied during Amphibian (*Bufo bufo*) embryo development.[15-18] Between the developmental enzymatic profiles of both amphibian and fish embryo a remarkable difference can be noted. For example, unlike fish, in amphibian embryo CAT activity was found to increase markedly throughout development, whereas a slight decrease of GST activity occurs at the end of development. In each case, the most noticeable difference observed between fish and amphibian embryo is that in the latter a significantly higher content of CAT, GSH-Px, GSSG-Rx and GST is present. The reason for these differences are at present unknown. However, it is possible that the high content of detoxifying enzymes present in the *Bufo bufo* embryo may be preparative to the transition from aquatic to terrestrial life; moreover in the *Bufo bufo* embryo very active melanogenesis occurs that may result in a higher oxygen and other cytotoxic species release.[34]

GSH-Px, GSSG-Rx, GLX I, GST, CAT and SOD activities were also measured in different trout tissues (Table 1). The highest activity of SOD, GLX I and GSSG-Rx was found in kidney, whereas the highest activity of both CAT and GST was found in liver followed by kidney and heart. It has to be noted that the GST, GLX I and GSSG-Rx activity values of both adult heart and muscle tissues were found to be lower than that present at the fry stage. The results obtained also indicate that the concentration of GSH-Px in trout tissues is extremely low compared with CAT. This confirms the primacy of CAT over GSH-Px also in the salmon tissues. Similarly, in guinea-pig tissues,[35] CAT, is by far the most important enzyme in removing H_2O_2. In other mammals, including man, both CAT and GSH-Px operate for the reduction of H_2O_2. Thus, species-variations in the strategies for

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TABLE 1
Glutathione transferase, glyoxalase I, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase activities in trout tissues

Tissue	Activity (mU/mg)						
	GST	GLX	GSSG-Rx	GSH-Px		SOD	CAT
				(H_2O_2)	(CHP)		
Young liver	290 ± 17	24 ± 2.0	18 ± 1.4	11 ± 0.91	13 ± 0.98	–	–
Adult liver	320 ± 19	16 ± 0.9	18 ± 1.3	12 ± 0.98	14 ± 1.1	3300 ± 215	225000 ± 9500
Kidney	170 ± 15	110 ± 9.1	70 ± 6.2	n.d.	n.d.	6300 ± 590	21000 ± 1950
Heart	28 ± 2.2	56 ± 4.8	10 ± 0.88	14 ± 1.1	17 ± 1.4	600 ± 58	3000 ± 250
Muscle	20 ± 1.6	45 ± 4.2	3.7 ± 0.29	1.3 ± 0.11	1.7 ± 0.12	1400 ± 120	1030 ± 95

n.d., not detectable in our experimental condition.

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removing toxic products of oxygen metabolism exist. On the other hand, our results are consistent with those reported by Aksnes and Njaa,[36] who found GSH-Px activity to be less than one thousandth that of CAT in several tissues of a number of fish species. Furthermore, in trout tissues GSH-Px activity value for cumene hydroperoxide is essentially identical to that obtained with H_2O_2. This means that despite the presence of substantial GST activity, only the Se-dependent GSH-Px enzyme is present in trout tissues. On the contrary, in the black bullhead liver, both the selenium-dependent and the selenium-independent GSH-Px forms are presents.[37]

Multiple forms of GST have been evidenced in all species so far investigated, including fish.[38-40] To investigate whether, in *Salmo iridaeus* the expression of GST subunits is modified with the passage from the embryonic to the adult life, the liver, heart, muscle and embryonic (stage 30–33) GST activity was first isolated through the GSH-affinity matrix and then analyzed by SDS-PAGE and HPLC techniques. The GSH-affinity purified materials from both embryos and adult fish tissues produced essentially an identical electrophoretic pattern showing two bands with an apparent molecular mass of about 23 and 25 kDa. When subjected to immunoblotting experiments with antisera raised against a number of human, rat and mouse enzymes belonging to alpha, mu and pi class GST, a positive cross-reaction between the major fish GST band (23 kDa) and the pi class GST antisera was noted (not shown). The present results are in accordance to those of George *et al.*[39] and Dominey *et al.*[41] who have demonstrated that the major GST form present in a number of fish species belongs to Pi class. No cross-reaction was seen between the minor fish GST band (25 kDa) and the alpha, mu and pi class GST. The electrophoretic result seems to indicate that no dramatic changes in the GST subunits expression occurs in fish with the passage from the embryonic to the adult life. The HPLC analysis of GST fractions purified from embryos and adult fish tissues strongly support this conclusion. In fact, in embryos, as well as in liver, muscle and heart, even thought in different amounts (Table 2) at least five peaks eluting respectively at min 32.29 (peak 1), 34.37 (peak 2), 36.26 (peak 3), 40.15 (peak 4) and 43.33 (peak 5) were found (Figure 2). The GST fraction eluting at 36.26 min (peak 3) cross-reacted with antisera raised against the Pi class GST. These results are different from those found in Amphibia.[18,20] In this latter, the HPLC GST subunit pattern of embryo was found to be remarkably different from that in toad tissues.[18,20] In particular, in the toad liver and kidney is present a GST isoenzyme (GST-7.64) not expressed during embryo development, which is highly active against

TABLE 2
Relative contribution of each GST subunit isolated by HPLC to the total GST content of Salmo iridaeus embryo and adult trout tissues

		GST subunit content (%)			
h.p.l.c. peak retention time	Class	Embryo	Heart	Liver	Muscle
32.39	−a	18.9 ± 3	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.3
34.37	−a	18.9 ± 4	4.4 ● 1	1.9 ± 0.7	2.5 ± 1
36.26	pi	15.5 ± 4	61.8 ± 8	72.8 ± 8	75.0 ± 8
40.15	−a	21.9 ± 2	31.4 ± 4	9.6 ± 2	13.0 ± 2
43.33	−a	24.8 ± 3	0.9 ± 0.3	15.0 ± 3	2.8 ± 0.9

a = no cross reaction was seen whith antisera raised against alpha, mu and pi class GST.

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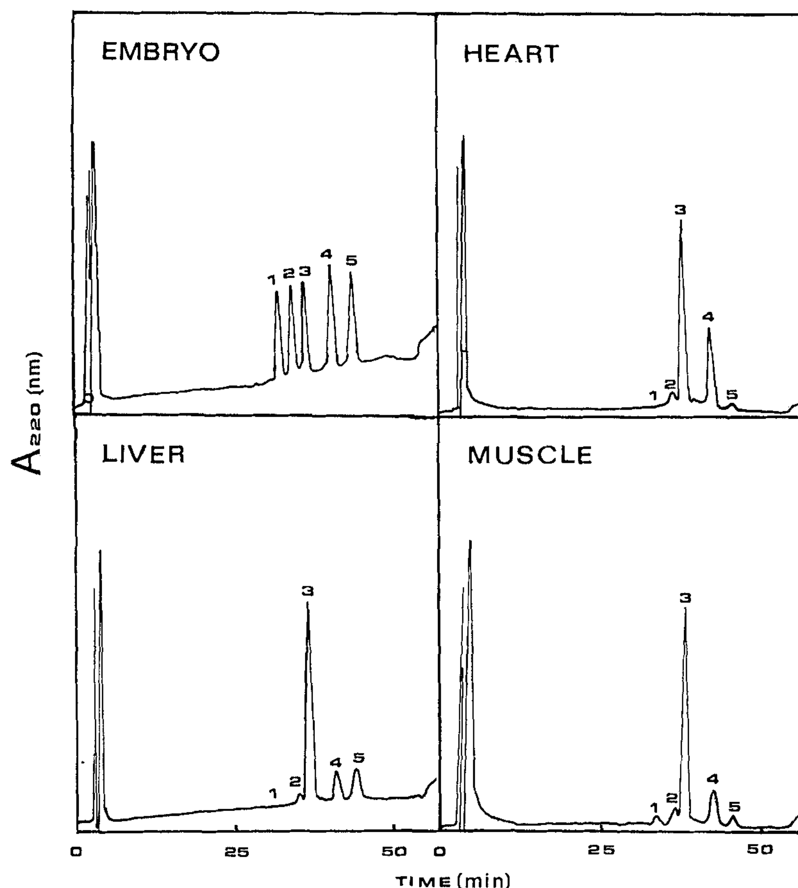

FIGURE 2 H.p.l.c. elution profile of *Salmo iridaeus* embryos, trout heart, liver and muscle glutathione transferase resolved by GSH-affinity chromatography.

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organic hydroperoxide.[18,20] Furthermore, toad liver is unable to express the major embryonic GST isoenzyme (GST-8.1).[18,20] In view of the Amphibian results the question arises whether, in the transition from the embryonic to the adult life, it is the different type of feeding or the change from the aquatic to the terrestrial environment that cause the switch from a GST pattern to another. The results obtained in fish strongly support the idea that the switch in the GST pattern that occurs in Amphibians, with the passage from the embryonic to the adult life, has to be attributed to the transition from an aquatic environment to a terrestrial one, rather than to a change in feeding physiology.

Acknowledgement

This work was supported by CNR Progetto Finalizzato "Prevenzione e controllo dei fattori di malattia" (FATMA); Sottoprogetto "Qualità dell'ambiente e salute." Contratto n. 91.001.146.PF41.

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References

1. B. Halliwell and J.M.C. Gutteridge (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, **219**, 1–14.
2. B. Chance, H. Sies and A. Boveris (1979) Hydroperoxide metabolism in mammalian organs. *Physiological Review*, **59**, 527–604.
3. R.A. Lawrence and R.F. Burk (1976) Glutathione peroxidase activity in selenium deficient rat. *Biochemical and Biophysical Research Communication*, **71**, 952–958.
4. J.R. Prohaska and H.E. Ganther (1977) Glutathione peroxidase activity of glutathione S-transferases purified from rat liver. *Biochemical and Biophysical Research Communication*, **76**, 437–445.
5. B. Mannervik and U.H. Danielson (1988) Glutathione transferases. Structure and catalytic activity. *CRC Critical Review Biochemistry*, **23**, 283–337.
6. D.L. Vander Jagt (1988) The glyoxalase system. In Glutathione: Chemical, Biochemical, and Medical Aspects (Edited by Dolphin D., Poulson R. and Avramovic O.) pp. 597-641. John Wiley and Sons, New York.
7. Thornalley P.J. (1990) Tho glyoxalase system: towards functional characterization and a role in disease processes. In *Glutathione: Metabolism and physiological functions*, (ed. J. Vina). CRC Press, Boca. pp. 135–143.
8. S.J. Stohs, W.A. Al-Turk and C.R. Angle (1992) Glutathione S-transferase and glutathione reductase activities in hepatic and extrahepatic tissues of female mice as functions of age. *Biochemical Pharmacology*, **31**, 2113–2116.
9. G.A. Hazelton and C.A. Lang (1983) Glutathione S-transferase activities in the yellow-fever mosquito [Aedes aegypti (Laisville)] during growth and aging. *Biochemical Journal*, **210**, 281–287.
10. E. Gerdin, O. Tyden and U.J. Eriksson (1985) The development of antioxidant enzymatic detence in the perinatal rat lung: activities of superoxide dismutase, glutathione peroxidase, and catalase. *Pediatric Research*, **19**, 687–691.
11. R.C. Strange, A.F. Howie, R. Hume, B. Matharoo, J. Bell, C. Hiley, P. Jones and G.J. Beckett (1989) The developmental expression of alpha-, mu-and pi-class glutathione transferase in human liver. *Biochimica and Biophysica Acta*, **993**, 186–190.
12. M.C. McElroy, A.D. Postle and F.J. Kelly (1992) Catalase, superoxide dismutase and glutathione peroxidase activities of lung and liver during human development. *Biochimica Biophysica Acta*, **1117**, 153–158.
13. L.B.J. Tee, K.S. Gilmore, D.J. Meyer, B. Kitterer, Y. Vandenberge and G.C.T. Yeoh (1992) Expression of glutathione S-transferase during rat liver development. *Biochemical Journal*, **282**, 209–218.
14. G.B. Principato, M. Bodo, M.G. Biagioni, G. Rosi and F.S. Liotti (1982) Glyoxalases and glutathione reductase activity changes in chicken liver during embryo development and after hatching. *Acta Embryologica Morphologica Experientia.*, **3**, 173–179.
15. C. Di Ilio, G. Del Boccio, M. Miranda, A. Manilla, O. Zarivi and G. Federici (1986) Glutathione peroxidase and glutathione reductase activities during *Bufo bufo* development. *Comparative Biochemistry and Physiology*, **83C**, 9–12.
16. G. Del Boccio, C. Di Ilio, M. Miranda, A. Manilla, O. Zarivi, A. Bonfigli and G. Federici (1987) Glutathione transferase activity during *Bufo bufo* development, *Comparative Biochemistry Physiology*, **86B**, 749–753.
17. M. Miranda, C. Di Ilio, G. Del Boccio, A. Bonfigli, O. Zarivi, A.M. Cimini, A. Poma and F. Amicarelli (1987) Glutathione S-transferase and catalase patterns and activities during Bufo bufo development. *Acta Embryologica Morphologica Experimenta*, **8**, 293–297.
18. A. Aceto, B. Dragani, P. Sacchetta, T. Bucciarelli, S. Angelucci, M. Miranda, A. Poma, F. Amicarelli, G. Federici and C. Di Ilio (1993) Developmental aspects of Bufo bufo embryo glutathione transferases. *Mechanism of Ageing and Development*, **68**, 59–70.
19. C. Di Ilio, A. Aceto, T. Bucciarelli, B. Dragani, S. Angelucci, A. Poma, F. Amicarelli, M. Miranda and G. Federici (1992) Glutathione transferase isoenzymes from Bufo bufo embryos at an early developmental stage. *Biochemical Journal*, **283**, 217–222.
20. A. Aceto, B. Dragani, T. Bucciarelli, P. Sacchetta, F. Martini, S. Angelucci, F. Amicarelli, M. Miranda and C. Di Ilio (1993) Purification and characterization of the major glutathione transferase from adult toad (*Bufo bufo*) liver. *Biochemical Journal*, **289**, 417–422.
21. J.M. Oppenheimmer (1961) *Experimental embryology*, Burges, Minneapolis.
22. P.C. Simons and D.L. Vander Jagt (1977) Purification of glutathione S-transferase from human liver by glutathione affinity chromatography. *Analytical Biochemistry*, **82**, 334–341.
23. U.K. Laemmli (1970) Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature*, **227**, 680–685.
24. H. Towbin, T. Stachelin and L. Gordon (1979) Electrophoretic transfer of protein from gels to

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nitrocellulose sheets: procedure and some applications. *Preceding National Academic Science*, USA, **76**, 4350-4354.
25. A.K. Ostlund-Farrants, D.J. Meyer. B. Coles, C. Southan, A. Aitken, P.J. Johnson and B. Ketterer (1987) The separation of glutathione transferase subunits by using reverse-phase high pressure liquid chromatography. *Biochemical Journal*, **245**, 423-428.
26. L.A. Del Rio, M. Gomez Ortega, A. Leal Lopez and J. Lopez Georgè (1977) A more sensitive modification of the catalase assay with the Clark oxygen electrode. *Analytical Biochemistry*, **80**, 409-415.
27. M. Sun and S. Zigman (1978) An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Analytical Biochemistry*, **90**, 81-89.
28. D.E. Paglia and W.N. Valentine (1967) Studies on the qualitative and quantitative characterization of erythrocytes glutathione peroxidase. *Journal Laboratory Clinical Medicine*, **70**, 158-169.
29. C. Di Ilio, P. Sacchetta. M. Lo Bello, A.M, Caccuri and G. Federici (1986) Selenium independent glutathione peroxidase, activity associated with cationic forms of glutathione transferase in human hearth. *Journal of Molecular and Cellular Cardiology*, **18**, 983-991.
30. C. Di Ilio, G. Polidoro, A. Arduini, A. Muccini and G. Federici (1983) Glutathione peroxidase, glutathione reductase, glutathione S-transferase and g-glutamyltranspeptidase activities in human early pregnancy placenta. *Biochemical Medicine*, **29**, 143-148.
31. W.H. Habig and W.B. Jakoby (1981) Assitys for differentiation of glutathione S-transferases. *Methods in Enzymology*, **77**, 398-405.
32. B. Mannervik, A.C. Aronsson, E. Marmstal and G. Tibellin (1981) Glyoxalase I (rat liver). *Methods in Enzymology*, **77**, 297-301.
33. M.M. Bradford (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248-254.
34. M. Miranda, F. Amicarelli, A. Bonfigli, D. Botti, O. Zarivi and A. Poma (1991) Changes of lipo-melanosome membrane leakage versus pH, charge and composition. *Melanoma Research*, **1**, 195-200.
35. S. Himeno, A. Takekawa and N. Imura (1993) Species difference In hydroperoxidase-scavenging enzymes with special reference to glutathione peroxidase in guinea-pigs. *Comparative Biochemistry and Physiology*, **104B**, 27-31.
36. A. Aksnes and L.F. Njaa (1981) Catalase, glutathione peroxidase and superoxide dismutase in different fish species. *Comparative Biochemistry and Physiology*, **69B**, 893-896.
37. J.F. Heisinger and S.M. Dawson (1983) Effect of selenium deficiency on liver and blood glutathione peroxidase activity in the black bullhead. *Journal Experimental Zoology*, **225**, 325-327.
38. P.I.N. Ramage and I.A. Nimmo (1984) The substrate specificities and subunit compositions of the hepatic glutathione S-transferases of rainbow trout (*Salmo Gairdneri*). *Comparative Biochemistry and Physiology*, **78B**, 189-194.
39. S. George, G. Buchanan, I.A. Nimmo and J.D. Hayes (1989) Fish and mammalian liver cytosolic glutathione S-transferases: substrate specificities and immunological comparison. *Marine Environmental Research*, **28**, 41-46.
40. T.K. Collier, S.V. Singh, Y.C. Awasthi and U. Varanasi (1992) Hepatic xenobiotic metabolising enzymes in two species of benthic fish showing different prevalences of contaminant-associated liver neoplasm. *Toxicology and Applied Pharmacology*, **113**, 319-324.
41. R.L Dominey, I.A. Nimmo. A.D, Cronshaw and J.D. Hayes (1991) major glutathione S-transferase in salmonid fish livers is homologous to the mammalian Pi-class GST. *Comparative Biochemistry and Physiology.*, **100B**, 93-98.

Accepted by Professor J.V. Bannister

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