Liver dehydrogenase levels in rainbow trout, *Salmo gairdneri*, fed cyclopropenoid fatty acids and aflatoxin B_1

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Abstract Cyclopropenoid fatty acids in the diet of rainbow trout caused significant reductions in liver protein and activity of glucose-6-phosphate dehydrogenase, NADP-linked isocitrate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase. Changes in total activity were usually accompanied by similar changes in specific activity. The activity of glucose-6-phosphate dehydrogenase appeared to be more sensitive to the ingestion of cyclopropenoid fatty acids than the other dehydrogenases studied. Feeding 20 ppb aflatoxin B_1 to rainbow trout did not significantly change the activity of the dehydrogenases except for a small increase in the activity of glucose-6-phosphate dehydrogenase after 21 days of feeding. Relationships of these changes to the cocarcinogenicity of cyclopropenoid fatty acids and the carcinogenicity of aflatoxin are discussed.

Supplementary key words dehydrogenases · cocarcinogen · fish · enzymes

Cyclopropenoid fatty acids (CPFA) have been reported to enhance the incidence and growth of aflatoxin-induced hepatoma in rainbow trout (1). Lee, Wales, and Sinnhuber (2) found similar results for purified sterculic acid. CPFA have also been shown to be cocarcinogenic with acetyl aminofluorene (3). Although CPFA were not carcinogenic to trout, histological examination revealed extreme liver damage, including deposits of glycogen and formation of fibers in liver parenchymal cells (3, 4). Livers from CPFA-fed trout were enlarged, firm, and displayed a lack of pigmentation. CPFA were deposited intact in all body tissues and accumulated with feeding time.

Presence of glycogen deposits in livers of CPFA-fed trout suggested that these cyclic fatty acids interfered with normal carbohydrate metabolism. To aid in understanding this phenomenon, the present study was undertaken to determine the effect of CPFA on several liver dehydrogenases involved in carbohydrate metabolism.

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*) of the Mt. Shasta strain were spawned, reared, and maintained as described by Lee et al. (5). Fingerlings were fed a semipurified test diet (5) for at least 90 days prior to initiation of the experiment. The trout were fed ad lib. 8 times a day throughout the growth period and experimental trials. Experimental diets were the control plus either 100 or 200 ppm CPFA derived from *Sterculia foetida* oil (50% sterculic and 5% malvalic acids) or 20 ppb aflatoxin B_1 (6).

The fish were killed by a sharp blow on the head. Livers were immediately removed, perfused with modified Krebs saline (7), and frozen in liquid nitrogen. Eight livers were combined and weighed, and a 1:5 (w/v) homogenate was made in 0.01 M Tris–citrate buffer (pH 7.0) in a micro container attachment (Eberbach Corp., Ann Arbor, Mich.) to a Waring Blendor for 1 min at a rheostat setting of 90. Time between death and homogenization was not more than 1 hr. The homogenate was centrifuged at 2500 *g* for 10 min at 4°C. 4 ml of this supernate was centrifuged at 100,000 *g* for 1 hr in a Beckman model L-2 refrigerated ultracentrifuge. The resulting supernate was used as enzyme source for the following studies.

Dehydrogenase activity was determined spectrophotometrically by measuring the change in cofactor (NADH or NADP) concentration at 340 nm (8). Considerable effort was made to optimize the assay conditions, and these conditions are presented in Table 1. Assay temperature was 25°C. Protein concentration of the supernate was determined by the ratio E_{280}/E_{260} according to Chaykin (9).

Abbreviations: CPFA, cyclopropenoid fatty acids; G-6-PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); NADP-ICDH, NADP-linked isocitrate dehydrogenase (EC 1.1.1.42); MDH, malate dehydrogenase (EC 1.1.1.37); LDH, lactate dehydrogenase (EC 1.1.1.27).

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TABLE 1. Dehydrogenase assay reaction mixtures

	MDH	LDH	G-6-PDH	NADP-ICDH
Tris–HCl buffer (M)[a]	0.022	0.026	0.032	0.030
pH	7.5	7.5	7.5	7.5
Substrate (M)				
Oxalacetic acid	8.03×10^{-4}			
Sodium pyruvate		1.6×10^{-3}		
Sodium isocitrate				7.21×10^{-6}
Sodium G-6-P			1.44×10^{-5}	
Cofactor (M)				
NADH	2.52×10^{-6}	4.45×10^{-6}		
NADP			3.96×10^{-4}	5.96×10^{-4}
Other additions (M)				
Mn^{2+}				1.6×10^{-2}
Mg^{2+}			1.596×10^{-4}	

[a] M, molarity.

All assays were performed in duplicate, and the means of these duplicates were used to calculate the data. Results presented in Table 2 reveal that the ultracentrifugation increased the specific activity of the enzymes three- to four-fold with good recovery. Therefore, this procedure was used throughout the study.

RESULTS AND DISCUSSION

In a preliminary trial in which 100 ppm CPFA was fed to 18-wk-old rainbow trout, protein, G-6-PDH, NADP-ICDH, and MDH contents of the supernate appeared to decrease after 13 days of feeding. This prompted an experiment in which 100 ppm CPFA was fed to 21-wk-old trout. These results are presented in Table 3. The soluble protein content of liver of CPFA-fed fish was lower than that of the controls, but the difference was significant only at the final sampling.

The activities of G-6-PDH and NADP-ICDH in preparations from cyclopropene-fed fish were lower than those of controls throughout the 38-day experiment (Table 3). LDH activity increased initially, returned to normal, and showed a large decrease after 13 days of feeding CPFA. Levels of MDH were below those of the controls in the 13–38-day samples. Changes in total activity were usually accompanied by similar changes in specific activity.

TABLE 2. Purification of dehydrogenases of rainbow trout liver by ultracentrifugation

Supernate	Activity	Protein	Specific Activity	Yield	Purification
	μmoles/min/ml	*mg/ml*	*μmoles/min/mg protein*	*%*	*fold*
Glucose-6-phosphate dehydrogenase					
2500 *g*	16.9	174	0.097		
100,000 *g*	16.9	46	0.368	100	3.8
NADP-isocitrate dehydrogenase					
2500 *g*	19.3	174	0.111		
100,000 *g*	16.9	46	0.368	88	3.3
Lactate dehydrogenase					
2500 *g*	164	174	0.94		
100,000 *g*	184	46	4.00	112	4.3
Malate dehydrogenase					
2500 *g*	1250	174	7.2		
100,000 *g*	1250	46	27.3	100	3.8

To determine the influence of dietary level of CPFA on the activity of the dehydrogenases, a diet containing 200 ppm CPFA was fed to 23-wk-old trout for 26 days. The results are presented in Table 4. Similar to the previous experiment, soluble protein decreased after 13 days on the CPFA diet. Again the activity of G-6-PDH was the most sensitive to feeding of CPFA, while NADP-ICDH level decreased at the second sampling period. LDH and MDH appeared to be more sensitive to the higher dietary level of CPFA and showed significant reductions in activity earlier than in the previous experiment.

Depression of enzyme activity, as demonstrated in these two experiments, could be due to many factors. Among these are repression of enzyme synthesis or partial inhibition of the enzymes. Decreased protein in the supernate may indicate that protein synthesis was decreased. However, an increase in protein catabolism would also account for this decrease in protein.

Fatty acid desaturase is inhibited by CPFA (10–12). Allen et al. (10) and Raju and Reiser (11) presented evidence that the mechanism of inhibition is the irreversible binding of enzyme sulfhydryl groups to the cyclopropene group of the CPFA. In vitro reaction between methyl sterculate and mercaptans has been demonstrated by Kircher (13). Yeast alcohol dehydrogenase (14) and castor bean lipase (15), which contain free sulfhydryl groups, are also inhibited by CPFA. In the present study, the reaction of CPFA with the sulfhydryl groups of the dehydrogenases could be the cause of the decrease in activity. The level of CPFA in liver and body tissues of trout increases with feeding time and dietary level (4). The accumulation of CPFA could account for the lower levels of dehydrogenase

TABLE 3. Effect of feeding 100 ppm CPFA on liver protein and dehydrogenases of 21-wk-old trout

Days of Experiment	No. of Fish	Protein	G-6-PDH Activity[a]	G-6-PDH Specific Activity[b]	NADP-ICDH Activity	NADP-ICDH Specific Activity	LDH Activity	LDH Specific Activity	MDH Activity	MDH Specific Activity
		mg/ml								
1–4	4	46.5 ± 0.3	13.0 ± 0.8[c]	0.27 ± 0.02[d]	17.5 ± 1.6	0.38 ± 0.03	212 ± 10[d]	4.5 ± 0.2[d]	1350 ± 41	28.5 ± 0.6
5–7	3	47.7 ± 0.9	11.2 ± 0.8[c]	0.24 ± 0.02[c]	16.0 ± 1.0[d]	0.34 ± 0.02[d]	194 ± 7	4.1 ± 0.1	1141 ± 105	23.7 ± 1.8
8–12	2	46.0 ± 2.0	12.0 ± 0.0[d]	0.26 ± 0.01[d]	19.0 ± 0.0	0.42 ± 0.01	202 ± 4	4.4 ± 0.0	1400 ± 50	30.0 ± 0.0
13–38	6	41.5 ± 2.5[d]	9.2 ± 0.4[e]	0.22 ± 0.01[e]	11.7 ± 1.2[e]	0.28 ± 0.02[e]	127 ± 7[e]	3.1 ± 0.1[c]	732 ± 61[e]	18.0 ± 1.8[e]
Control	10	48.1 ± 1.1	18.8 ± 1.0	0.39 ± 0.02	20.5 ± 1.0	0.43 ± 0.02	180 ± 6	3.8 ± 0.1	1262 ± 44	26.1 ± 0.9

[a] μmoles/min/ml ± SE.
[b] μmoles/min/mg protein ± SE.
[c] $P < 0.01$.
[d] $P < 0.05$.
[e] $P < 0.001$.

TABLE 4. Effect of feeding 200 ppm CPFA on liver protein and dehydrogenases of 23-wk-old trout

Days of Experiment	No. of Fish	Protein	G-6-PDH Activity[a]	G-6-PDH Specific Activity[b]	NADP-ICDH Activity	NADP-ICDH Specific Activity	LDH Activity	LDH Specific Activity	MDH Activity	MDH Specific Activity
		mg/ml								
1–4	4	46.5 ± 1.3	15.0 ± 0.9[c]	0.32 ± 0.01[c]	17.7 ± 0.8	0.38 ± 0.01	176 ± 9.6	3.8 ± 0.2	1098 ± 127	23.7 ± 2.5
5–7	2	44.5 ± 1.5	10.8 ± 1.2[d]	0.24 ± 0.03[c]	13.0 ± 1.0[c]	0.30 ± 0.02	137 ± 2.5[c]	3.1 ± 0.2	892 ± 72	20.5 ± 2.5
8–12	3	47.3 ± 1.3	12.3 ± 0.3[d]	0.26 ± 0.01[d]	12.9 ± 1.8[c]	0.27 ± 0.03[c]	105 ± 6.6[e]	2.2 ± 0.1[d]	707 ± 32[d]	15.0 ± 0.0[d]
13–26	2	38.5 ± 0.5[c]	10.0 ± 4.0[c]	0.27 ± 0.11	9.6 ± 2.4[d]	0.25 ± 0.06	73 ± 11.0[e]	1.9 ± 0.3[d]	386 ± 0[d]	10.0 ± 0.0[d]
Control	5	47.2 ± 1.8	18.6 ± 0.9	0.40 ± 0.03	18.7 ± 1.1	0.39 ± 0.03	167 ± 6.1	3.6 ± 0.2	1195 ± 73	25.2 ± 1.5

[a] μmoles/min/ml ± SE.
[b] μmoles/min/mg protein ± SE.
[c] $P < 0.05$.
[d] $P < 0.01$.
[e] $P < 0.001$.

TABLE 5. Effect of feeding 20 ppb aflatoxin B_1 on liver protein and dehydrogenases of 18-wk-old trout

Days of Experiment	No. of Fish	Protein	G-6-PDH Activity[a]	G-6-PDH Specific Activity[b]	NADP-ICDH Activity	NADP-ICDH Specific Activity	LDH Activity	LDH Specific Activity	MDH Activity	MDH Specific Activity
		mg/ml								
1–6	3	55.3 ± 1.2	20.6 ± 2.0	0.37 ± 0.03	26.3 ± 1.4	0.48 ± 0.03	198 ± 2.6	3.57 ± 0.13	1413 ± 117	25.3 ± 1.3
7–20	2	46.5 ± 5.5	18.0 ± 4.0	0.38 ± 0.03	18.0 ± 6.0	0.37 ± 0.08	163 ± 6.0	3.54 ± 0.29	1061 ± 96[c]	22.5 + 0.5
21–36	3	48.6 ± 1.4	24.0 ± 2.9	0.49 ± 0.04[c]	20.0 ± 1.0	0.41 ± 0.01	176 ± 6.2	3.64 ± 0.03	1268 ± 43	26.0 ± 0.7
Control	12	50.4 ± 1.3	19.4 ± 0.8	0.39 ± 0.01	23.2 ± 1.0	0.46 ± 0.02	185 ± 4.1	3.68 ± 0.11	1310 ± 33	26.0 ± 0.9

[a] μmoles/min/ml ± SE.
[b] μmoles/min/mg protein ± SE.
[c] $P < 0.05$.

activity at the longer feeding times and higher dietary level.

Cocarcinogenic activity of CPFA in trout has not been explained. Reaction of CPFA with sulfhydryl groups and the subsequent inhibition of enzymes may be important in understanding this cocarcinogenicity. Harington (16) hypothesizes that carcinogens may react with sulfhydryl groups involved in cell control and division, causing inhibition and a subsequent compensatory response leading to overproduction of synthesizing enzymes and eventual loss of control. In the present work, CPFA have been shown to decrease the activity of some dehydrogenases involved in carbohydrate metabolism in vivo. It is reasonable to believe that the activity of other enzymes may also be reduced.

Feeding of 20 ppb aflatoxin B_1 to 18-wk-old rainbow trout had very little effect on the activities of dehydrogenases studied (Table 5). The activity of MDH was decreased at the 7–20-day sampling period but returned to normal thereafter. Contrary to these observations, Lee (17) reported significant decreases in liver protein and LDH after injection of aflatoxin B_1 into rats.

Shankaran, Raj, and Venkitasubramanian (18) noted a decrease in the activities of UDP-glucose–glycogen transglucosylase, glycogen phosphorylase, phosphoglucomutase, and glucose-6-phosphatase and an increase in the com-

bined hexose monophosphate dehydrogenases of livers of chicks administered aflatoxin B_1. In agreement with this, results presented in Table 5 suggest that G-6-PDH activity and specific activity were increased by feeding aflatoxin B_1 to rainbow trout. This indicates that the hexose monophosphate shunt of carbohydrate metabolism may be enhanced by aflatoxin B_1 ingestion.

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