In vitro Hydrolysis of Polyoxyethylene Esters by Tissues of the American Eel and Atlantic Salmon

by D. J. WILDISH
Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.
and
D. BEATTY
Zoology Department, University of
Alberta, Edmonton, Alberta, Canada

Oil dispersants commonly consist of polyoxyethylene (POE) esters or POE ethers in an organic or aqueous solvent (Anon 1970). We have investigated the possibility that tissues of the Atlantic salmon *(Salmo salar)* and American eel *(Anguilla rostrata)* are able to hydrolyse POE esters. It has been suggested by Wildish (1972) that hydrolysis may detoxify POE esters and thus explain the greater acute toxicity of POE ethers.

Methods and materials

Salmon were hatchery-reared parr (15.5-16.2 cm, 34.5-39.3 g) or post-smolts (29.8-36.2 cm, 245.5-420.2 g). Silver eels (80-100 cm, 500-700 g) from the St. Croix River, New Brunswick, were used.

Samples for determination of esterase activity were prepared from weighed amounts of fresh or thawed tissue stored at -15 C. Samples of tissue (20-300 mg) were homogenized in 2 ml 0.7% w/v NaCl in a Sorvall Omnimixer immersed in an ice bath. The homogenate was washed into the reaction vessel with 10 ml 0.7% NaCl immediately. Acetone drying of tissues (Mahadevans et al. 1966) of known wet weight was carried out at -2 to -10 C. The powders obtained after grinding in a mortar and sieving in a 0.5 mm mesh stainless steel sieve were stored in a desiccator at -15 C or at laboratory temperature during one month's use without loss of activity. Blood serum was prepared by centrifuging whole heparinized blood at 12,000 g for 10 minutes.

Substrates were technical products (Table 1) supplied by the makers and used without further purification. POE chain length was checked in some of the compounds by infrared spectrophotometry (Zitko 1972). Solutions (2 ml) of the surfactants in distilled water were added to the reaction vessel and the volume adjusted to 16 ml with 0.7% w/v NaCl.

Activity of esterase (E.C. 3.1) was determined with the method of Jensen et al. (1959) by continuous titration of product acid with 0.01N NaOH at a constant pH and temperature. Apparatus consisted of a Radiometer titrator type TTT1c, Titrigraph type SBR2c, and an autoburette type ABU1b.

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TABLE 1

Nonionic surfactants used in esterase activity determinations

Chemical name	F.W.	Trade name	Supplier
POE(4) monolaurate	376	Pegosperse 200 ML	Glyco Chemicals Inc.
POE(9) monolaurate	596	Pegosperse L-9	Glyco Chemicals Inc.
POE(14) monolaurate	816	Hallco CPH-43	C.P. Hall Co.
POE(14) dilaurate	998	Kessco TM	Armour Industrial Chemicals
POE(9) mono-oleate	678	Varionic Polyglycol esters 400 MO	Northern Petrocemical Co.
POE(4) lauryl ether	363	Ethosperse LA-4	Glyco Chemicals Inc.

Reaction velocity was determined over the initial 16 min of the progress curve and expressed as millimoles substrate hydrolysed/min/g of fresh or dry weight or as millimoles/min/ml of blood fraction. Corrections for spontaneous non-specific acid production in tissues or non-enzymatic hydrolysis of substrate were made as necessary. Precision of the method was ± 10%(± S.D.). In substrate concentration studies where the same weight of tissue was used for each determination, precision was ± 5%.

Results

Specific esterase activities of acetone-dried tissues of post-smolt salmon (1% w/v POE(9) monolaurate, pH = 8.5, T = 25.0°C) showed that maximum activity occurred in the liver (5.02 millimoles/min/g). Hydrolytic rates for other tissues were: intestine 34%, spleen 29%, and muscle only 6% that of liver. No activity was found with pyloric caecae and brain tissue, indicating either absence of the enzyme or that the determination is limited by the sample preparation method. Frozen plasma hydrolysed 2.82 and fresh plasma 2.22 millimoles/min/ml under the same conditions.

No activity was found with acetone-dried eel and post-smolt salmon liver and POE(4) lauryl ether, indicating that any enzymatic reaction did not yield acidic products.

The specific esterase activity of fresh and acetone-dried liver is shown in Table 2.

TABLE 2

Specific esterase activity of liver (millimoles/min/g) of salmon and eel. Substrate = 0.5% w/v POE(9) monolaurate, pH = 8.5, T = 25.0°C.

Fish	Wet weight g	Dry weight g	Esterase activity Fresh	Esterase activity Acetone-dried
S. salar, parr				
1	0.4019	0.0221	1.15	3.91
			1.15	3.75
2	0.3440	0.0276	1.50	4.76
			1.40	4.97
3	0.3011	0.0320	1.89	6.63
			1.78	5.88
Mean			1.48	4.98
A. rostrata				
1	4.9271	0.4706	4.88	21.15
			3.81	19.35
2	5.1773	0.6203	7.96	29.60
			9.10	28.40
3	4.0736	0.7178	5.85	14.38
			5.82	14.15
Mean			6.24	21.17

Fresh eel liver is able to hydrolyse POE(9) monolaurate at a mean rate 4.2 times that of salmon parr liver. Comparing the acetone-dried preparations the mean hydrolytic rate is also 4.2 times greater in eel liver, indicating no differential effect of acetone treatment.

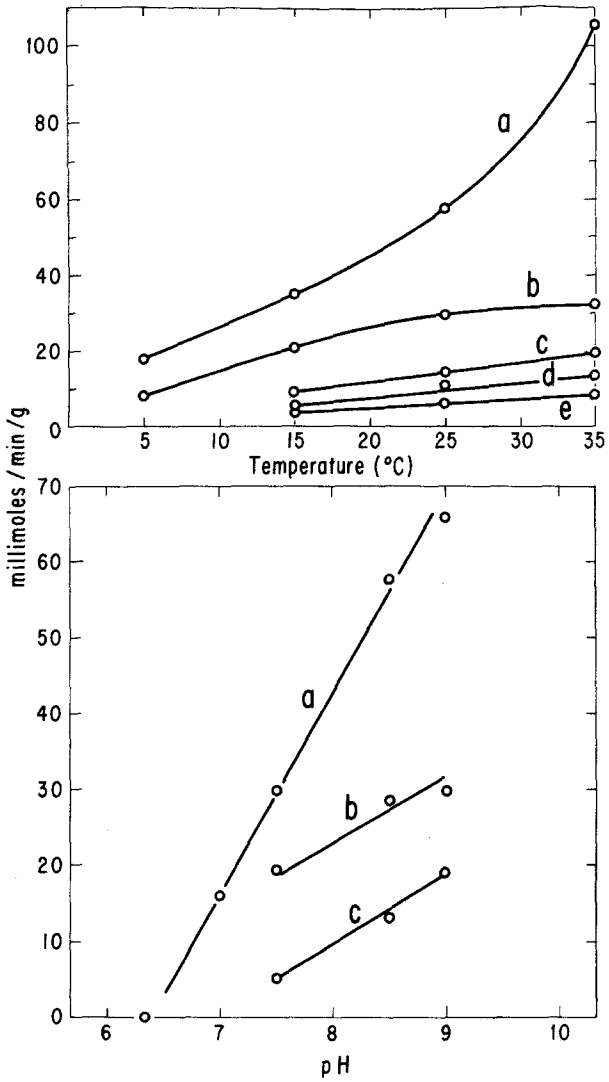

Figure 1. Effect of temperature (pH = 8.5) and pH(T = 25.0°C) on esterase activity. (a) POE(4) monolaurate; (b) POE(9) monolaurate; (c) POE(14) monolaurate; (d) POE(14) dilaurate; (e) POE(9) mono-oleate. Concentration = 1% w/v.

An acetone-dried eel liver (fresh weight = 17.70 g, dry weight = 2.93 g) was used to determine the pH and temperature dependence of hydrolysis (Fig. 1). The hydrolytic rate is linearly related to pH with the slope

steeper for POE(4) monolaurate than the other compounds tested. No temperature optima were found in the range 5-35 C. The temperature dependence of hydrolysis of the POE esters varied between compounds (see Fig. 1).

Maximum velocities (V) and Michaelis-Menten constants (Km) were determined from plots of v against $\frac{v}{s}$ and fitted linear regression lines

TABLE 3

V and Km for acetone-dried eel liver, pH = 8.5, T = 25.0 C

Substrate	V millimoles/min/g	Km
POE(4) monolaurate	59.25	7.02×10^{-2}
POE(9) monolaurate	29.69	7.45×10^{-2}
POE(14) monolaurate	14.54	9.09×10^{-2}
POE(14) dilaurate	12.47	8.19×10^{-2}
POE(9) mono-oleate	6.49	5.40×10^{-2}

Conclusions

The liver of Atlantic salmon and American eel hydrolyse POE esters at a rate dependent on POE chain length. Increasing POE chain length decreases V, possibly determined by the rate of breakdown of the enzyme/substrate complex. Substituting oleic for lauric acid in POE(9) ester results in decreased V and Km.

Compared with the activity of acetone-dried beef liver (unpublished observations) hydrolytic rates are approximately 8 to 32 times less with eel and salmon liver and POE(14) monolaurate as substrate.

If hydrolysis of POE esters does lead to partial detoxication in fish, it follows from this work that eels should be less susceptible to poisoning by POE esters than salmon and that the hydrolytic detoxication becomes progressively faster as the homologous series is descended. Both predictions are subject to other factors involved in poisoning such as uptake and excretion dynamics.

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