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Effect of an Anionic Detergent on the Lipid Moieties of Various Cell Types in the Opercular Epidermis of *Rita rita*

Debasish Roy

Department of Zoology, Banaras Hindu University, Varanasi–221 005, India

With the rise of industrial civilization, the use of synthetic detergents as cleansing agent in India has become so wide spread that it has even replaced the traditional soap. This extensive use has created havoc by polluting the whole of the aquatic ecosystem and threatening the Government for its immediate remedies. Various reports concerning the effects of synthetic detergents on different organ systems of freshwater and marine fishes have been reviewed by Abel (1974). But very less attention has been given on the histopathology of epidermis (Bardach et al. 1965; Pohla-Gubo and Adam 1982; Roy 1987) especially the change in the cytology and cytochemistry influenced by the detergent. Zaccone et al. (1985),however, studied the pattern of change of activity of few enzymes of the epidermis of Heteropneustes fossilis under the influence of an anionic detergent, sodium alkyl benzene sulphonate (LAS)

Biological membranes are mainly composed of lipids and proteins to which carbohydrates may be covalently bound and it is probable that the detergent may come in contact with the membrane and exert its influence. Therefore, the aim of this investigation has been to examine the histochemical change caused by an anionic detergent, dodecylbenzene sodium sulfonate in the various lipid moieties of different cell types in the opercular epidermis at different time intervals of exposure.

MATERIALS AND METHODS

Fish, Rita rita (14± 2 cms in length) were collected from riverside of Ganges at Varanasi,U.P. during January and kept for 30 days in optimum laboratory conditions for acclimatization. Static bioassay test was performed to determine 96-hr LC50 value of an anionic detergent, dodecylbenzene sodium sulfonate (C_{12}-LAS) obtained from Koch Light Laboratories, Colnbrook, England, to the fish following the methods of APHA,AWWA,WPCF (15th edn.). 80 fish were divided into 8 batches having ten fish in each batch and were kept in aquaria of size (60cms x 30 cms x 45 cms) containing 30 litres of tap water to avoid crowding. The tap water used was having pH 7.1 ± 0.3, hardness 158 mgs/litre (as $CaCO_3$), dissolved oxygen

Send reprint requests to D.Roy,464,Saheednagar,Bhubaneswar,India.

6.5 to 8.4 ppm and temperature 21 $\pm$ 0.8^{o}C. The first batch exposed to normal tap water only was treated as control and all others as experimental. In all the experimental aquaria, 207 mgs of detergent per aquarium i.e. for 30 litres of water at the rate of 6.9 mgs/litre (96-hr LC50 of the detergent) were dissolved in a small amount of water and properly mixed in the aquaria. Care was taken to avoid froathing. Dead fish,as soon as detected were at once discarded. Control as well as experimental fish were sacrificed at frequent intervals (15min, 30min, 45min, 1 hour, 2h, 3h, 6h, 12h, 1 day, 2d, 3d, 4d, 5d, 6d, 7d, 8d) . Opercula of the fish (right side only) were dissected out and fixed in 10% neutral formalin and formal calcium for 6 hours. Frozen sections were cut at 10-15 μm using cryostat (AO model 849C) at-30^{o} C and were mounted on clean glass slides and dried for 10-15 minutes at room temperature (20-25^{o} C) before subjecting them to histochemical techniques for the demonstration of various lipid moieties. For controls, sections were also subjected to different extration procedures following Bayliss High (1982) in order to confirm the staining reactions.

RESULTS AND DISCUSSION

With Sudan black B with and without prior treatment of various extraction techniques, the epithelial cells throughout the opercular epidermis indicate the presence of moderate amount of lipids. The intensity of Sudan black B colour enhances greatly after bromine water treatment. This colour intensity markedly gets reduced with prior acetone treatment. Prior chloroform methanol treatment completely extracts the lipid contents of these cells which also give moderate reaction for neutral lipids with Oil red O. Prior acetone treatment both at room temperature and at 4^{o}C completely inhibits the Oil red O staining of these cells. Relatively a weak reaction for phospholipids is observed with acid haematein technique which too is extracted by chloroform-methanol treatment with and without 1% HCl and 4% H_2O. With nile blue sulphate, the epithelial cells stain bluish purple which becomes light blue if treated with acetone prior to treatment with stain. Prior chloroform methanol treatments with or without 1% HCl or 4% H_2O donot cause fading of this colour reaction with this technique. These cells also contain small amount of fatty acids, cholesterol and cholesterol esters as evidenced by moderate reaction with various techniques (Table 1).

Transferring the fish into the detergent medium for three or more days causes disappearance of most of the lipid moieties from the epithelial cells as evidenced by negative reactions with most of the lipid staining techniques. Such condition prevails throughout the period of the experiment. However, only neutral lipids could be located in these cells upto 5 days of detergent treatment, after which the reaction for neutral lipids is lost also,suggesting complete disappearance of all the types of lipid moieties from the epithelial cells of the opercular epidermis.

The secretory products of the goblet mucous cells,distributed mainly in the outer layer of the opercular epidermis remain unstain-

Table 1. A summary of the histochemical changes in the lipid constituents of the epithelial in the opercular epidermis of Rita rita at various intervals of detergent exposure.

Chemical Constituents	Histochemical techniques	Colour symbol	Epithelial cells (after days of exposure)																		
			Outer layer							Middle layer							Basal layer						
			C	1	2	3	4	5	6to8	C	1	2	3	4	5	6to8	C	1	2	3	4	5	6to8
General lipids	Sudar black B	Blb	+	+	+	+	±	±	–	+	+	+	±	±	–	–	+	+	+	±	±	–	–
	Acetone/Sudan black B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Bromine/Sudan black B	B	++	++	++	+	+	+	–	++	++	++	+	+	±	–	++	++	++	+	±	–	–
	Chloroform–methanol with & without 1% HCl or 4% H_2O/ Sudan black B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Neutral lipids	Oil red O	R	+	+	+	+	+	±	–	+	+	+	+	+	±	–	+	+	+	+	+	+	–
	Acetone/Oil Red O	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Differentiation of neutral and acidic lipids	Nile blue sulphate	Pur	+	+	+	±	±	±	–	+	+	+	+	+	±	–	+	+	+	+	+	+	–
	Acetone/Nile blue sulphate	Bl	+	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–
	Chloroform–methanol with & without 1% HCl or 4% H_2O/Nile blue sulphate	Bl	+	–	–	–	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	–
Phospholipids	Bromine/acetone/ Sudan black B	Blb	+	+	+	–	–	–	–	+	+	+	±	–	–	–	+	+	±	–	–	–	–
	Chloroform–methanol with & without 1% HCl or 4% H_2O/Bromine/acetone/ Sudan black B	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Acid haematein	Bl	±	±	±	±	–	–	–	±	±	±	±	–	–	–	±	±	±	±	–	–	–
	Chloroform–methanol with & without 1% HCl or 4% H_2O/acid haematein	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 1 Continued

A summary of the histochemical changes in thelipid constituents of the epithelial in the opercular epidermis of Rita rita at various intervals of detergent exposure.

Chemical Constituents	Histochemical techniques	Colour symbol	Epithelial cells (after days of exposure)																		
			Outer layer							Middle layer							Basal layer						
			C	1	2	3	4	5	6to8	C	1	2	3	4	5	6to8	C	1	2	3	4	5	6to8
Free fatty acids	Copper rubeanic acid	G	+	±	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetone/Copper rubeanic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cholesterol & cholesterol esters	Perchloric acid naphthoquinone(PAN)	P	+	±	-	-	-	-	-	+	±	±	-	-	-	-	+	+	±	-	-	-	-
	Cold acetone/PAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free cholesterol	Digitonin/PAN	P	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
	Digitonin/acetone/PAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triglycerides	Calcium-lipase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetone/ calcium-lipase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Symbols: Bl,blue; BLb,bluish black; G,green; P,pink; R,red; Pur,purple;-,negative; ±, weak; +,moderate; ++,strong reaction. C, control conditions

ed with most lipid stains except with nile blue sulphate which stains the contents purple which is acetone labile.The peripheral areas of these cells stain bluish black and deep black with Sudan black B and bromination/Sudan black B techniques and appear red with Oil red O which gets extracted by prior acetone and chloroform-methanol treatment with and without 1% HCl or 4% H_2O treatments. The peripheral areas of the goblet mucous cells also stain blue with nile blue sulphate and/with copper rubeanic acid for free fatty acid, bluish black with acid haematein for phospholipids and pink with PAN for cholesterol esters which are extracted by various extraction procedures applied in this study. The secretory contents, however, remain unstained for triglycerides.

3 days of detergent treatment induces a decrease in most of the lipid moieties in the peripheral regions of the goblet mucous cells. However, with acid haematein test a weak reaction for phospholipids persists upto 5 days after which no reaction for any of the lipid moieties could be observed till the end of the experiment. The secretory contents, like those of the control condition, remain unstained throughout.

The perinuclear area and the boundaries of the club cells,distributed throughtout in the opercular epidermis stain positively with Sudan black B that is intensified by prior bromination, and stain purple with nile blue sulphate. When stained with these methods after prior extraction (Table 2), these cells show negative reaction. These areas give weak reactions with copper rubeanic acid technique for free fatty acids, with acid haematein test for phospholipids and with PAN test for cholesterol and cholesterol esters. The contents of the club cells remain mostly unstained with various histochemical techniques applied in this study.

The intensity of reaction for general lipids with Sudan black B and Bromine/Sudan black B, for acidic and neutral lipids with nile blue sulphate, for free fatty acids with copper rubeanic acid gets weaker and only a weak reaction is observed upto 2 days after the fish are transferred to the detergent medium. After this period, the intensity of reaction further decreases and after 5 days,no reaction for these lipid moieties could be observed.

This study experiences a decreases in various lipid moieties of the epithelial cells, goblet mucous cells and club cells under the influence of the detergent treatment. The effect of the detergent treatment on the mucous membrane is instant as evidenced by immediate profuse secretion by the cells of the epidermis (Roy 1987). The mucous coat offers a quick and first barrier to prevent the penetration of the toxicity of the detergent. This coat is, however, not sufficient nor is a permanent one but synthesis and secretion of mucus is a continuous process.

The toxicity of a detergent is a function of both its surface activity and chemical toxicity (Abel 1974). He further explained that reduced surface tension might modify the sequence of events during acute poisoning but definitely not the only cause of

Table 2. A summary of the histochemical changes in the lipid constituents of the gland cells in the opercular epidermis of Rita rita at various intervals of detergent exposure.

Chemical Constituents	Histochemical techniques	Colour symbol	Gland cells (after days of detergent exposure)													
			Goblet mucous cells							Club cells						
			C	1	2	3	4	5	6to8	C	1	2	3	4	5	6to8
General lipids	Sudan black B	Blb	+[b]	+[b]	±[b]	±[b]	±[b]	-	-	+[c,d]	±[c,d]	±[c,d]	-	-	-	-
	Acetone/Sudan black B	-	-[b]	-[b]	-[b]	-[b]	-[b]	-[b]	-	-[c,d]	-[c,d]	-[c,d]	-	-	-	-
	Bromine/Sudan black B	B	++[b]	++[b]	+[b]	+[b]	+[b]	+[b]	-	++[c,d]	+[c,d]	±[c,d]	-	-	-	-
	Chloroform-methanol with & without 1% HCl or 4% H_2O/Sudan black B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Neutral lipids	Oil red O	R	+[b]	+[b]	±[b]	-	-	-	-	+[c,d]	+[c,d]	+[c,d]	-	-	-	-
	Acetone/Oil red O	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Differentiation of neutral and acidic lipids	Nile blue sulphate	Pur	±[a]	±[a]	±[a]	-	-	-	-	+[a]	±[a]	±[a]	-	-	-	-
		Bl	++[b]	++[b]	+[b]	-	-	-	-	-	-	-	-	-	-	-
	Acetone/Nile blue sulphate	Bl	+[b]	±[b]	-	-	-	-	-	-	-	-	-	-	-	-
	Chloroform-methanol with & without 1% HCl or 4% H_2O/Nile blue sulphate	Bl	+[b]	±[b]	-	-	-	-	-	-	-	-	-	-	-	-
Phospholipids	Bromine/acetone/Sudan black B	Blb	+[b]	-	-	-	-	-	-	+[c,d]	+[c,d]	-	-	-	-	-
	Chloroform-methanol with & without 1% HCl or 4% H_2O/Bromine/acetone/ Sudan black B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 Continued

A summary of the histochemical changes in the lipid constituents of the gland cells in the opercular epidermis of Rita rita at various intervals of detergent exposure.

Chemical Constituents	Histochemical techniques	Colour symbol	Gland cells (after days of detergent exposure)													
			Goblet mucous cells							Club cells						
			C	1	2	3	4	5	6to8	C	1	2	3	4	5	6to8
	Acid haematein	Blb	+[b]	+[b]	+[b]	±[b]	±[b]	±[b]	-	±[c,d]	±[c,d]	±[c,d]	-	-	-	-
	Chloroform-methanol with & without 1% HCl or 4% H_2O/acid haematein	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free fatty acids	Copper rubeanic acid	G	+[b]	+[b]	-	-	-	-	-	±[c,d]	±[c,d]	±[c,d]	-	-	-	-
	Acetone/Copper rubeanic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cholesterol & cholesterol esters	Perchloric acid naphthoquinone(PAN)	P	±[b]	-	-	-	-	-	-	±[c,d]	-	-	-	-	-	-
	Cold acetone/PAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Free Cholesterol	Digitonin/PAN	P	±[b]	-	-	-	-	-	-	-	-	-	-	-	-	-
	Digitonin/ acetone/PAN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triglycerides	Calcium-lipase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetone/calcium lipase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Symbols: Bl,blue; Blb,bluish black; G,green; P,pink; R,red; Pur,purple;-,negative; ±,weak; +,moderate; ++,strong;reactions a,secretory contents; b,periphery; c,perinuclear area; d,cellboundary. C,control condition

disruption.Biological membranes constitute extremely complex mixture of lipids,protein ions etc.. In spite of greater complexities of membranes, it seems clear to us that the general principles of detergent action apply to all as well. There will be modifying factors due to special structural features of biological membranes. It is believed that the lytic process in haemoglobin as described by Helenius & Simon (1975) may also be operating in this case being a resultant of the interaction between the surfactant and the lipids of the membrane.

Although, the question of skin permeability to water is complex (Isaia 1984;Rankin & Bolis 1984) and the structure of the epidermis is probably more important from this point of view than the chemistry of the secretions, the lipid in the skin surface secretions may provide a barrier between the internal and the external environment of the fish, acting as a water repellent and limiting the entry of water into the body of these species.

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