Short communication report

SIALIC ACID AND SIALIDASE PROFILES OF THE MOSQUITO *CULEX QUINQUEFASCIATUS* SAY 1823 IN SAMARU, ZARIA, NIGERIA

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Sialic acid is 9-carbon carboxylated monosaccharides with a general structure similar to N-acetyl neuraminic acid. It is one of the most important molecule of life since it occupy the terminal position on macromolecules and cell membranes and is also involved in many biological and pathological phenomenon (Schauer 2001) as demonstrated in the dual role it plays in the protection and adaptation of life by micro-organisms who use it to promote infection (Cornfield *et al.* 1981; Schauer 2001). However, many aspects of the regulation of their metabolism at the enzyme and gene level as well as their function remain uncertain (Schauer 2001).

Sialidase, otherwise called neuraminidase, is the enzyme that liberate sialic acid from the macromolecules (Schauer 2001). It has been found to play key roles in viral and bacterial infections (Muller 1974) and has also been reported to be a significant factor in the transmission of *Trypanosoma brucei* Plimmer & Bradford 1899 in mosquitoes in the laboratory (Sallau *et al.* 2004). Although the exact mechanism for the role of sialic acid in mosquitoes is not well described, there is the need to study the concentrations of free and total sialic acid at different stages of the developmental cycle of *Culex* mosquito to elucidate the presence of sialic acid in each stage. Relating sialic acid concentration to the infection biology of the mosquito vector at the various developmental stages could pave ways for better understanding the role of sialic acid in mosquitoes. This paper reports on the sialic acid and sialidase profiles in the *Cx. quinquefasciatus* developmental stages in Zaria Nigeria.

Mosquito larvae were collected from polluted water habitats in Samaru, Nigeria and brought to the Department of Biological Sciences, Ahmadu Bello University Zaria for identification and rearing. The larvae were kept in containers placed inside a cage and fed with yeast supplements until adult emergence. Identification was according to taxonomic keys of (Hopskins 1952; Service 1980). The adult females that emerged were fed in-vivo on restrained pigeon whose feathers on the flank were removed. A bowl of water in an earthen pot was placed in the cage for oviposition.

The male mosquitoes were fed with 7 % sucrose solution in a 10ml jar placed in the cage. Oviposited eggs were removed after 2-3 days and placed in 100 ml plastic container into which 50 ml of declorinated water was introduced. The whole container was then kept at room temperature for the production of the eggs raft, larva, pupa and adult stages that were used for the investigation.

The mosquito developmental stages used in the investigation were in the following proportions. Five batches of eggs, 50 each of 1st, 2nd, 3rd, and 4th instar larvae, 50 pupae and 50 adult females. Prior to the assay, each developmental stage was washed with distilled water and macerated in 1000µl distilled water in 1.5 ml eppendorf tube. Hydrolysis was preceded by adding 2000 µl of 0.1N HCL and incubated at 80 ^{0}C in a water bath for 1 hr. Total sialic acid concentration was then determined by taking readings in a spectrophometer at a wavelength of 549 nm.

Sialic Acid Concentration Assay
Free sialic acid concentration was determined by the Thiobarbituric Acid Method according to Schauer (1978). The process was carried out by oxidizing 500 µl of the hydrolyzed sample in 250 µl of peroxidate solution and incubated in a water bath at 37 ^{0}C for 30 min. 100 µl of Sodium arsenite was added to the mixed solution to neutralize the excess peroxidate solution, after which 1000 µl of Thiobarbituric Acid was added and heated in a water bath at 100 ^{0}C for 8 min to form a coloured complex, at which point, 2500 µl of Butanol Acetic Acid (19:1) was added to the solution to extract the sialic acid. The solution was centrifuged at 5 rpm for ten minutes and optical readings at 549 nm in the spectrophometer and recorded, the actual concentrationsof sialic acid was extrapolated from the standard curve (Schauer 1978; Sallau *et al.* 2001).

Sialidase – Enzyme Assay
10µl of fetuin (1mg/ml) was added to 500 µl of a hydrolyzed sample and incubated at 37 ^{0}C for 1hr. Optical readings of sialidase was obtained from the spectrophotometer at 549 nm and the concentration was determined by extrapolation from the standard curve (Sallau *et al.* 2001). Both the sialic acid and sialidase assays were run in triplicates.

The absorbance values obtained from the spectrophotometer (Table 1) were used to determine the concentrations of sialic acid and sialidase (Table 2) of the development stages of the mosquito from the regression equation of the Standard Curve. The means and standard errors of sialic acid and sialidase concentrations in the different stages of development of the mosquito are presented in Figure 1.

The sialic acid and sialidase absorbance readings of the developmental stages of the mosquito taken at 549 nm using the spectrophotometer are presented in Table 1 while the concentrations are presented in Table 2.

The result show that the amount of sialic acid vary with developmental stages of *Cx. Quinquefasciatus,* being lowest in

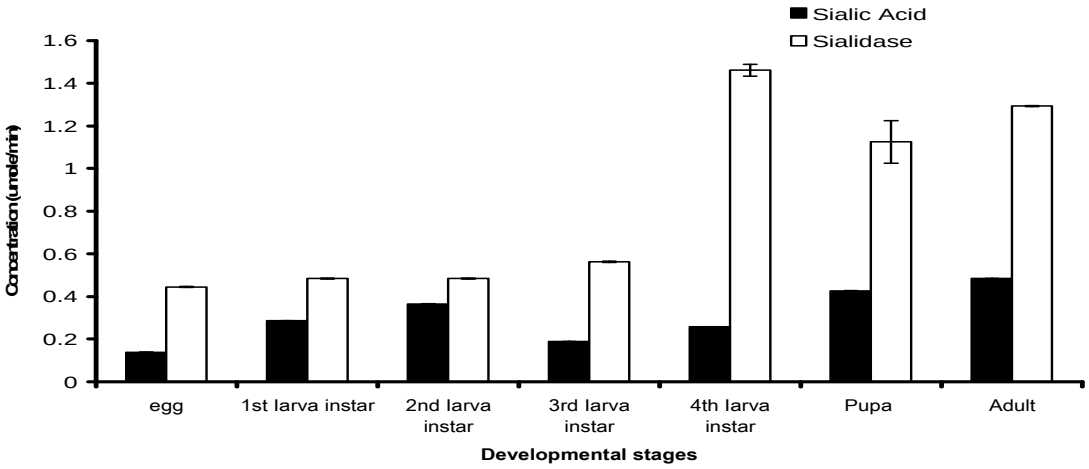

FIG 1: SIALIC ACID AND SIALIDASE CONCENTRATIONS OF DEVELOPMENTAL STAGES OF CULEX QUINQUEFASCIATUS.

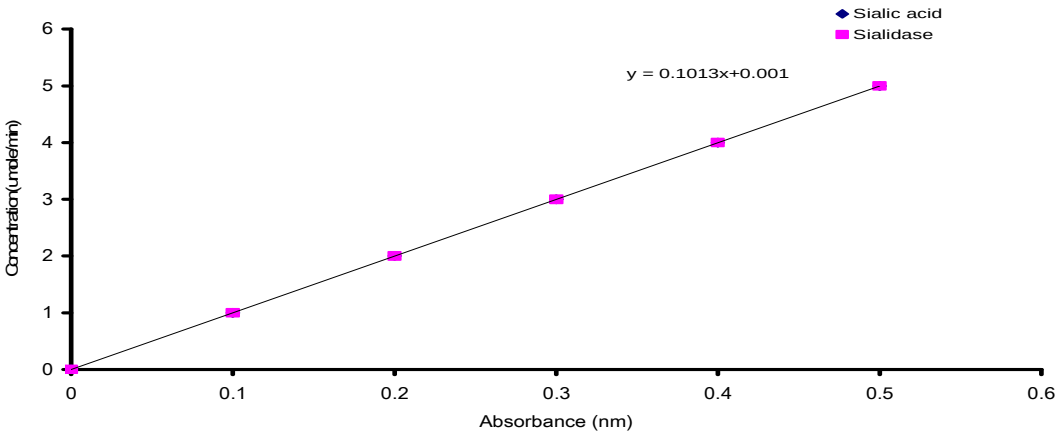

FIG.2. STANDARD CURVE FOR SIALIC AND SIALIDASE

TABLE 1. SIALIC ACID AND SIALIDASE ABSORBANCE OF DEVELOPMENTAL STAGES OF *CX. QUINQUEFASCIATUS*

Developmental Stages	Absorbance (594 nm)	
	Sialic acid	Sialidase
Egg	0.015	0.046
1st larval instar	0.030	0.050
2nd larval instar	0.038	0.050
3rd larval instar	0.020	0.058
4th larval instar	0.027	0.149
Pupal	0.044	0.115
Adult	0.050	0.132

the egg stage and highest in the adult stage. Similarly, the amount of sialidase was lowest in the egg stage and highest in 4th larva instar (Fig. 1 and Table 2).

TABLE 2. SIALIC ACID AND SIALIDASE CONCENTRATIONS OF DEVELOPMENTAL STAGES OF *CX. QUINQUEFASCIATUS*

Developmental Stages	Concentration (µmoles/min)	
	Sialic acid	Sialidase
Egg	0.138	0.444
1st larval instar	0.286	0.484
2nd larval instar	0.365	0.484
3rd larval instar	0.188	0.563
4th larval instar	0.257	1.461
Pupal	0.425	1.125
Adult	0.484	1.293

The presence of sialic acid and sialidase in all the developmental stages indicates that the two compounds are natural macromolecules of the insect which may be necessary for adaptations during the development of the mosquito from egg to adult stages as reported by Schauer (2001). Sialic acid has also been reported in *Drosophila melanogaster* (Schauer 2001) which is also an insect. The presence of higher quantity of sialic acid observed in the adult mosquito than the other immature stages may signify the additional relevance of the compound at this stage of the insect for activities such as flight, mating and some aspects of transmission of pathogens. Similar role of sialic acids have been reported in the mid-guts of *Cx. pipiens pipiens* infected with *Trypanosoma congolense* Broaden 1904 (Sallau *et al*. 2004).

The higher levels of sialidase recorded than those of sialic acid in all the developmental stages of the mosquito may be due to the presence of other trans-sialidases which catalyses the transfer of sialic acids among a variety of molecules without utilizing CMP-sialic acid (Varki 1992; Scudder *et al.* 1993). The high level of sialidase in 4th instar larva and not in the adult stage suggests that sialic acid might have been degraded to pyruvic acid and N-acetyl mannosamine by pyruvate sialylated lyases. A more detailed comparative study on the levels of sialidase on the development of the insect is recommended on a wider scale.

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REFERENCES

Corfield, A.P.; Michalski, J. C. & Schauer, R. (1981). The substrate specificity of sialidases from micro-organisms ans mammals. In: *Perspectives in Inherited Metabolic Diseases* (G. Tettamanti, P. Durand & S. Donate, eds.), Vol. 4 Edirmes, Milan, Italy

Hopkins, G. H. E. (1952). *Mosquitoes of the Ethiopian region. Larval bionomics of mosquitoes and the taxonomy of Culicine Larvae*, the British Museum (Natural History), London.

Muller, H. E. (1974). Die Pathogrenestiche Beeting Inikrobieller Neuraminidasen stsch. *Deutsche Medizinische Wochenschrift* 99: 1933-1940.

Sallau, A.U.; Nok, A. J.; Ndams, I. S. & Balogun, E. O. (2004). Role of sialic acid in the mid-guts of *Trypanosoma congolense* infected *Culex pipiens pipiens* mosquitoes. *African journal of Biotechnology*, 3(8): 405-408.

Schauer, R. (1978). *Characterization of sialic acid in methods in enzymology*. Vol 50c (Ginsburg, V. Ed.) Academic press New York. San Francisco.

Scudder, P.; Doom, J. P.; Chuenkova, M.; Manger, I. D. & Pereira, M. E. (1993). Enzymatic characterization of B-D-galactoside & 2,3 trans-sialidase from *Trypanosoma cruzi*. *Journal of Biological Chemistry*. *268*: 9886-9891

Service, M.W. (1980). A guide to medical entomology. The Macmillan Press Ltd. London.

Varki, A. (1992). Diversity in Sialic acids *glycobiology* 2:25-40