International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 7, Issue 2, pp: 1-5, 2022
https://doi.org/10.55126/ijzab.2022.v07.i02.001
http://www.ijzab.com

Review Article

THE STRUCTURAL AND FUNCTIONAL EVALUATION OF AMYLASE ACTIVITY IN *DROSOPHILA MELANOGASTER*, IN COMPARISON TO OTHER RELATED INSECT SPECIES: A DETAILED APPROACH

[1*]Aparajita Chakraborty and [2]Md. Masiur Rahman

[1]Department of Biotechnology, St. Xavier's College, Kolkata -700016, India
[2]Department of Zoology, Raja Peary Mohan College, Uttarpara,Kolkata-712258, India

Article History: Received 20th January 2021; Accepted 22th February 2021; Published 6th March 2021

ABSTRACT

The role of *Drosophila melanogaster*, or fruit fly, in biological research has been highly remarkable, particularly in genetics and developmental biology. It is a valuable model organism for research for almost a century which holds a significant impact on human health. One of the remarkable features of this organism recently identified is the ability of the larvae to excrete amylase. Amylase, a glycosyl hydrolase is responsible for the degradation of α-1,4 glycosidic bonds in starch molecule to its constituent monosaccharides. The excretion of such a digestive enzyme by *Drosophila* larvae was predicted to play an important role in the physical softening of food and improvement of efficiency of food ingestion. Apart from *Drosophil*a, certain insects such as bugs, silkworm, honeybee, or even other species of the *Drosophila* family are known to secrete amylases, which may differ in properties such as optimum pH, temperatures or protein sequences as reported by the presence of several amylase gene copies. Various studies had been performed on the various 'isozymes' of such amylases to determine their catalytic activities, similar heat sensitivities, or temporal expressions but the exact role of such enzymes in the human system is yet to be elucidated.

Keywords: Amylases, Catalytic activities, Drosophila, Gene copies, Insects.

INTRODUCTION

Drosophila Melanogaster has been identified as a model creature in various fields of biological research and is known by various names for its various attractions: fruitfly, pomace fly and vinegar fly. It is known over the winter in storage areas, where it can eat or spoil a lot of food, by laying eggs on immature fruit (Haloi *et al*., 2014; Henrissat *et al*., 2002; Baker *et al*., 1980). The main reasons why such a body is used in various targeted applications. Human diseases lie in the fact that many basic biological, physical and emotional properties are stored between mammals and *D. melanogaster* and that about 75% of human genetic disease is believed to have an active homologue in fruitfly. Not only that, it is no longer needed and there are many such factors that make it ideal for studying research on animal development and behavior, neurobiology and many other types of diseases and human genetic conditions.

One notable feature to be noted is the liquid enzyme amylase, (α- 1,4-glucan-4-glucanohydrolases, EC 3.2.1.1) which transports starch of hydrolyses and other polysaccharides to its maltose monosaccharides, maltotriose, and residual branched maltodextrins as final products (Figure 1) This enzyme is secreted not only by Drosophila species but also by many other insects from *Bombyx mori,Apis mellifera*, *Apis dorsata*, *Locusta migratoria, Aedes aegypti, Anopheles gambiae* etc (Da Lage, 2018 and Da Lage *et al*., 2000). It is therefore considered to be one of the basic enzymes required for the hydrolysis of carbohydrates, acquired mainly during the stage of survival as it is vital during that particular stage. Therefore, many α-amylase inhibitors are found in many plants including the common bean, *Phaseolus Vulgaris*, which plays a key role in the natural defense of these starch-eating insects (Haloi *et al*., 2014). In the next update, we will share an overview of the importance of the amylase

*Corresponding Author: Aparajita Chakraborty, Department Of Biotechnology, St. Xavier's College, Kolkata -700016, India, Email: aparajitachak95@gmail.com, Mobile: +91 9674036903.

enzyme activity present in *Drosophila Melanogaster* and other insects. The role of such an enzyme in Drosophila is believed to be responsible for maintaining the digestion of food in humans, but whether other enzymatic-acting insects have a significant effect is still unknown.

Amylase activities of *Drosophila melanogaster* and other insect species

Amylase: A major Digestive Enzyme

Alpha amylase (α-1,4-glucan-glucanohydrolase, EC 3.2.1.1) is a cell-rich enzyme that down regulates gluc-1,4 glucosidic interactions of starch and other endogenous products and produces oligosaccharides. Another type of amylase is β-amylase which is also made up of bacteria, fungi, plants and is responsible for clearing the second α-1,4- glycosidic compound, removing two units of sugar (maltose) at a time. Both types of amylase are present in seeds. The third type of amylase, or γ-amylase is responsible for the purification of α-1,6 glycosidic bonds and the final α-1,4 glycosidic bond that ultimately does not reduce amylose and amylopectin but produces glucose (Saini *et al*., 2017; Affifi *et al*., 2008; Wang *et al*., 2011). It has a very strong activity at an acidic pH of about 3. Alpha-amylase can be found in a variety of sources such as plants, animals and bacteria and holds a variety of functions in the bread, chocolate, paper industries and even in the biofuels industry. It is a faster digestive enzyme as compared to β-amylase. The ideal pH is 6.7-7.0 while the maximum temperature is found at about 37^{O}C. The addition of iron ions such as Ca^{2+}, Mg^{2+}, Mn^{2+} was found to increase enzyme activity and Hg^{2+}, Na+, K+, $Cu^{+2,}$ Fe^{+2} and Zn^{+2}. Were responsible for inhibiting the activity of protease (Saini *et al*., 2017, Affifi *et al*., 2008). Nowadays a variety of bacterial and fungal amylases are widely used in industrial use due to their low cost, consistency, short duration and local need for production and ease of process and improvement.

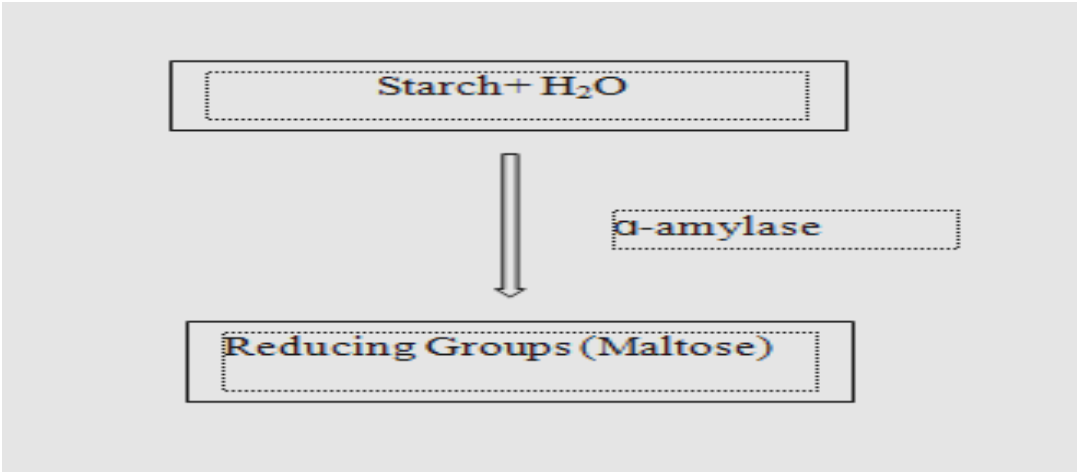

Figure 1.α-amylase degrades starch to reducing sugar maltose

Amylase activity in *Drosophila Melanogaster*

An important feature of *Drosophila Melanogaste*r is the release of amylase enzyme responsible for the external digestion of their food. Various experiments were performed to determine the activity of amylase to investigate whether their external digestive power differs in response to changes in the external environment, one of which is the iodine starch agar (ISAM) method. Using this particular method it is observed that the amount of amylase released during the larvae increases with increasing agar concentration, while previous studies of changes in the larval growth rate by the addition of amylase to food showed that pupation occurred approximately 24 hrs later in this food-containing diet with 1% amylase compared to those without amylase. Such results show that the larvae are able to regulate their release of amylase by responding to changes in the external environment and their level has a significant impact on the growth rate of the larvae (Sakaguchi and Suzuki, 2013). An important feature of the *Drosophila Melanogaster* worms that should be noted is their ability to facilitate external digestion by binding to the production and production of digestive enzymes - a substance called 'social digestion'.

The amylases of other insects

The feature of amylase activity is seen not only in the case of *Drosophila Melanogaster*, but also in other insects. Such enzymes have long been studied for scientific purposes and used in insects; In many such species alpha-amylases are produced by many copies of the gene (Figure 2) .They are found mainly in the pit, and enzymological parameters are found to vary between the various insects. Coleopteran molecules have excellent acidic activity, dipteran amylases have a neutral bias while lepidodipterans have an alkaline taste (Da Lage, 2018, Da Lage *et al*., 2000). Without looking at the copy number, it was originally believed that

phytophagous insects should have more amylolytic enzymes compared to carnivorous insects; Different vegetable diets were responsible for different controls of amylase levels. The common characteristics present in all insect amylases are of the same size i.e. the coding sequence is found around 1500 nucleotides, corresponding to a mature weight of approximately 50 to 55kDa proteins, after the release of signal peptide, as the enzyme is secreted . The alternative can be found in some way, as in the case of mosquitoes where the long N-terminal domain is located. Insect amylases are almost identical to animal amylases; assigned to a small GH13_15 family of glycosyl hydrolases and other inorganic enzymes and vertebrate amylases below GH13_24, which is a specific subclass of implants (Da Lage, 2018).

A certain amino acid extension called the 'flexible hoop' and the GHGA motif, which emerges near the catchment have been reported missing in a series of insect amylase sequences. For example, the GHGA motif was not available thus leading to a reduction in the flexible loop in most Coleopteran sequences, with the exception of two of them. In the case of Hymenopteran genres, it was found that two groups of groups lived together, one group with a loop, and the other group did not. In the Muscomorpha flies, the Amyrel group does not have a GHGA feature that suggests that the recurring loss of the dynamic group may be due to selective barriers to evolution, which remains to be determined. Another outstanding feature shown in evolution, is the incorporation of stored arginine into glutamine into other unrelated amylases; The presence of this arginine is responsible for the preparation of active chloride ions that modify protein conversion and in the absence of any harmful bridge formation occurring. (Da Lage, 2018; Sakaguchi and Suzuki, 2013). The presence of glutamine residues has been found in all types of Lepidopteran, as well as amyrel proteins of other drosophilids, with the exception of *Drosophila Melanogaster*. Therefore, this was an important point of difference between the amylase of *Drosophila Melanogaster* containing the enzymes of other insects. Glutamine-containing amylases fail to bind chloride ions, but are still active, chloride- independent due to various variations suggested to be in proper alignment with the alkaline pH in the central cavity. However, differences have been found in the case of Amyrel acting on a neutral pH thus contradicting the independent view of chloride at high pH values. Some types have been reported with specific pH optima, either due to different tissue specifications, or stage specifications. The high pH of lepidopteran species may be a condition for feeding tannin-rich plants as high pH is associated with a decrease in the binding of tannins to healthy proteins and improved digestion (Da Lage *et al*., 2000 ; Benkel and Hickey, 1986).

Other dipper species such as *Drosophila Melanogaste*r and *Ceratitis Capitata* have amylases with pH values of 7.4 and 8.0 respectively. Hymenopteran species such as *Monomorium pharaonis* and *Apis mellifera* have pH enzymatic values of 5.0-5.5 respectively, while coleopteran species *Acanthoscelides obtectu*s have a pH of about 7-7.5. Some lepidopteran species such as *Manduca sexta* and *Austinia nubilali* have enzymatic optimum pH values of 10 and 11 respectively. The high pH values for certain related species are listed in (Table 1). High temperatures vary for different *Drosophila Melanogaster* and other related species, but are highly dependent on testing as long periods of incubation at high temperatures can accelerate enzymerelease (Wang *et al*., 2011). For example, the positive temperatures of *Drosophila Melanogaster, D. sechell*a and *D. Erecta* amylases 37^{O}C is one of the immature fragments but 57^{O}C to 60^{O}C using pure in vitro enzymes. It is thought that species living under the sun in open fields should have more hot amylases compared to cold-lived species; however it is not easy to determine the temperature of these amylases without enzyme protocols or assays.

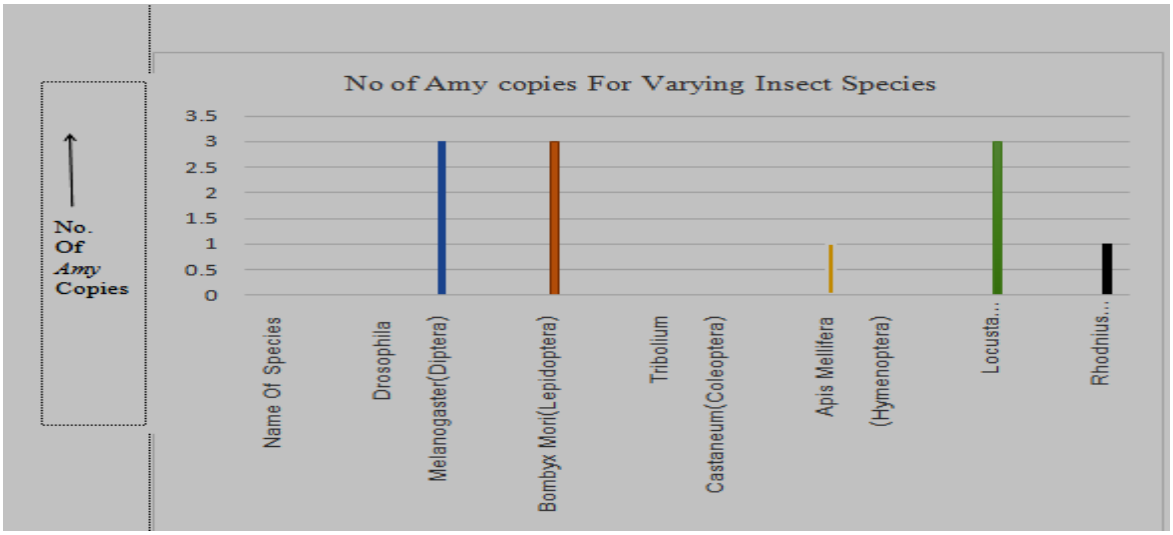

Figure 2. No. of Amy Copies for Each Related Insect Species.

Table 1. List of optimum pH for amylases of some insect species.

Order	Species	Optimum pH
Hemiptera	*Leptoglossus zonatus*	5.6
Hemiptera	*Aphis fabae*	7
Coleoptera	*Acanthoscelides obtectus*	7-7.5
Coleoptera	*Cryptolestes ferrugineus*	5.0-5.5
Diptera	*Drosophila melanogaster*	7.4
Diptera	*Aedes aegypti*	7.0
Hymenoptera	*Monomorium pharaonis*	5.0-5.5
Hymenoptera	*Apis mellifera*	5.0-5.5
Lepidoptera	*Manduca sexta*	10
Lepidoptera	*Ostrinia nubilalis*	11

Applications of Amylase from Drosophila/ Insect Species

As mentioned earlier, *Drosophila Melanogaster* larvae and bacteria can cause digestion by removing enzymes from the external environment. Many 'Drosophilists' have noticed that the surface of non-seeded foods tends to be slightly wet and soft with the growth of such larvae. Therefore, as people digest food after putting it in their mouths, it has been accurately predicted that the digestive enzymes, namely amylases can physically digest human-absorbed food and thus improve digestive function. In addition, softening such food appears to be a great help to such caterpillars so that they can roam the food (Benkel and Hickey, 1986, Kikkawa, 1964). The release of amylase by other species of insects appears to have a similar application, though it has not yet been clear. The role of such digestive enzymes in insects has been investigated; more research studies are being conducted on it.

CONCLUSION

The role of *Drosophila Melanogaster* has long been known to researchers, owing to various factors such as small size, easy growth in laboratories, presence of 4 pairs of chromosomes and most importantly possessing a 44% similarity to human chromosomes, making it a useful tool in many human neurodegenerative diseases. One important feature of this insect is the secretion of digestive enzymes, or amylases which holds a valuable role in the digestion of starch by the respective cleavage of the α-1,4 glycosidic bonds of its corresponding amylose subunits into simpler monosachharides. There are mainly three forms of amylases which have been detected in such species such as α- , β- and γ- amylases; β-amylases are responsible for the cleavage of the second α- 1,4 glycosidic bond, thereby cleaving off two glucose subunits(maltose) at a time, while the third form γ-amylase cleaves the α-1,6 glycosidic bond and the α-1,4 glycosidic bond at the non reducing end of amylose and amylopectin to yield glucose molecules. Such alpha amylases possess a maximum activity at an optimum pH and temperature, just like the other enzymes and even hold various industrial applications ranging from bread-making or chocolate factories. There are various assays to detect the presence of this particular enzyme in the gut of Drosophila and other insects, one of them being the iodine starch agar method (ISAM). Drosophila being a dipteran species possesses an amylase enzyme exhibiting maximum activity at an optimum pH of 7.4 and a temperature of 37^{O}C which is due to the fact that all dipteran species possess a neutral optimum pH and temperature. Like Drosophila, *Ceratitis Capitata* is a dipteran species exhibiting amylase activity at pH 8.0. Coleopteran species such as *Acanthoscelides obtectus* possess enzymes which are active at an acidic optimum pH, while lepidopteran species such as *Manduca sexta* and O*strinia nubilali* are found to possess amylases showing activities at pH 10 and 11 respectively, owing to their alkaline preferences. The course of evolution has been found to play a major role for such related insect species which may be revealed by various features such as the presence of a certain amino acid stretch called the flexible hoop, along with the motif GHGA which may be shortened for some coleopteran species due to the absence of the motif. In case of some Hymenopteran species, a certain group may possess the loop while the other group do not. Another interesting feature to be noted is the substitution of an arginine residue by glutamine residue in most species, except for *Drosophila Melanogaster* which are chloride-independent. The presence of such amylases in Drosophila species holds a major role in the softening of the foods taken up by humans and thereby improving the efficiency of food digestion. For other related insect species, these digestive enzymes are believed to play a similar role, but yet their detailed applications for mankind is yet to be fully elucidated in the modern, progressing world of research.

ACKNOWLEDGEMENT

This is a review article, so the authors drafted the manuscript by collecting data from various research papers and created tables.

REFERENCES

Affifi AF, Kamel EM, Foaad MA, Fwwzi EM, Housay MM (2008).Purification and characterization of alpha

amylase from *Pencillium olsonii* under the effect of some antioxidants vitamins. *Global Indian Journal of Biotechnology*. 3(1):14-21.

Baker, J. E., & Woo, S. M. (1985). Purification, partial characterization, and postembryonic levels of amylases from Sitophilus oryzae and *Sitophilus granarius*. *Archives of Insect Biochemistry and Physiology*, 2(4), 415-428.https://doi.org/10.1002/arch.940020409.

Benkel, B. F., & Hickey, D. A. (1986). Glucose repression of amylase gene expression in *Drosophila melanogaster*. *Genetics*, 114(1), 137-144.https://doi.org/10.1093/genetics/114.1.137.

Da Lage, J. L. (2018). The amylases of insects. *International Journal of Insect Science*, 10, 1179543318804783.https://doi.org/10.1177%2F1179543318804783.

Da Lage, J. L., Maczkowiak, F., & Cariou, M. L. (2000). Molecular characterization and evolution of the amylase multigene family of Drosophila ananassae. *Journal of Molecular Evolution*, 51(4), 391-403. https://doi.org/10.1007/s002390010102.

Drosophila melanogaster. Animal Diversity Web. (2022). Retrieved 19 January 2022, from https://animaldiversity.org/accounts/Drosophila_melanogaster/.

Haloi, D. J., Hasan, I., & Boro, N. (2014). Evaluation of amylase activity in fruit fly, *Drosophila melanogaster* and the inhibitory effect of common bean, *Phaseolus vulgaris* extract. *Journal of Entomology and Zoology Studies*, 2(6), 95-98.

Henrissat, B., Deleury, E., & Coutinho, P. M. (2002). Glycogen metabolism loss: a common marker of parasitic behaviour in bacteria. *Trends in Genetics*, 18(9), 437-440.https://doi.org/10.1016/S0168-9525(02)02734-8.

Hoorn AJ, Scharloo W. (1980) Functional significance of amylase polymorphism in *Drosophila melanogaster*. III. Ontogeny of amylase and some alpha-glucosidases. *Biochemistry Genetics*. (1-2):51-63. https://doi.org/10.1007/BF00504359.

Kikkawa, H. (1964). An electrophoretic study on amylase in Drosophila melanogaster. *The Japanese Journal of Genetics*, 39(6), 401-411. https://doi.org/10.1266/jjg.39.401.

Saini, R., Saini, H. S., & Dahiya, A. (2017). Amylases: Characteristics and industrial applications. *Journal of Pharmacognosy and Phytochemistry*, 6(4), 1865-1871.

Sakaguchi, H., & Suzuki, M. G. (2013). Drosophila melanogaster larvae control amylase secretion according to the hardness of food. *Frontiers in Physiology*, 4, 200. doi: 10.3389/fphys.2013.00200.

Wang, S. L., Liang, Y. C., & Liang, T. W. (2011). Purification and characterization of a novel alkali-stable α-amylase from *Chryseobacterium taeanense* TKU001, and application in antioxidant and prebiotic. *Process Biochemistry*, 46(3), 745-750.https://dx.doi.org/10.1016%2Fj.procbio.2010.11.022.