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INVITRO ASSAY FOR THE SPECIFIC ACTIVITY OF HUMAN SALIVARY AMYLASE ON STARCH

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ABSTRACT

Objective: is to estimate the specific activity of starved and unstarved human salivary amylase on starch invitro.

Materials and methods: Saliva collected from starved and unstarved humans subjected to Protein estimation and hydrolytic activity on starch.

Results and discussion: Experimental evidences suggested that, 1ml of starved human saliva contains more (74µg) protein than unstarved human saliva (60 µg). Starved human saliva hydrolysed starch in to maltose more (400mg) than unstarved human saliva (250mg). According to this invitro study, the Specific activity of 1 ml of starved salivary amylase is (1.0 micro mole/mg /min) higher than the unstarved human ptyalin (0.771µ mole/mg-1/min-1) on starch.

Conclusion: The saliva of starved humans has higher ptyalin and specific activity than unstarved humans.

KEYWORDS : Ptyalin Starch Specific activity Saliva

INTRODUCTION

Starchy substances constitute the major part of the human diet for most of the people in the world, as well as many other animals. Starch molecules are glucose polymers linked together by the alpha-1, 4 and alpha-1, 6 glucosidic bonds. In order to make use of the carbon and energy stored in starch, the human digestive system, with the help of the enzyme amylases, must first break down the polymer to smaller assimilable sugars, which is eventually converted to the individual basic glucose units. An unbranched, single chain polymer of 500 to 2000 glucose subunits with only the alpha-1, 4 glucosidic bonds is called *amylose*. On the other hand, the presence of alpha-1, 6 glucosidic linkages results in a branched glucose polymer called *amylopectin*.

In human physiology, although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which having its own isoform of human α-amylase. Salivary amylase or ptyalin is the digestive enzyme of the saliva which is secreted by the salivary glands. This enzyme digests boiled starch into dextrins and maltose. Dextrins are also formed as an intermediate product during hydrolysis of starch. It works best at an optimum pH 6.8 and optimum temperature 37oC. It is activated by chloride ions. During hydrolysis of starch; amylodextrin, erythrodextrin and maltose formed, which are detected by the colours such as blue, purple, red and no colour, respectively, by the addition of dilute iodine solution. The specific activity of an enzyme per milligram of total protein (expressed in µmol min−1mg−1). Specific activity gives a measurement of the activity of the enzyme. It is the amount of product formed by an enzyme in a given amount of time under given conditions per milligram of total protein. Specific activity is equal to the rate of reaction multiplied by the volume of reaction divided by the mass of total protein. The SI unit is katal kg−1, but a more practical unit is µmol mg−1 min−1. Enzyme activity is a measure of the quantity of active enzyme present and is thus dependent on conditions, which should be specified.

Health benefits of saliva analysed in the year 2005[1]. Studies on the proteins of human saliva conducted[2]. In 1993 conducted study on the parotid glands of rats to demonstrate the effect of starvation on the amylase secretion. According to this study, salivary amylase secreted from the glands of rats starved for 24h showed diurnal variation, with two peaks at 13 and 21hours. The peak at 21 hour was more notable than that in fed rats, because of the accumulation of a large amount of amylase in the tissue due to starvation[3]. A study on human salivation and its applications to psychophysiology was conducted[4].

MATERIALS AND METHODS

The saliva collected from starved and unstarved humans subjected to proteins estimation by standard method[5]. The hydrolytic activity of salivary amylase quantified by prescribed procedure[6,7].

RESULTS AND DISCUSSION

The results indicated that 1ml of starved human salivary amylase contains 74 µg of protein (ptyalin), which hydrolyses starch in to 400µg of maltose (reducing sugar) in 30 minutes. The enzyme activity is always expressed in micromoles of product/mg protein /min .i.e., called specific activity (specific activity is the unit of enzyme activity/mg protein/min). So 1mg of salivary amylase hydrolysed starch in to 30 µ moles in 30 minutes. This indicated that the specific activity of salivary amylase in starved human is 1 µ moles/mg/min (table 1)

According to table 1, 1ml of unstarved human salivary amylase contains 60µg of protein, which hydrolyses starch in to 200µg of glucose in 30 minutes. So 1mg of salivary amylase hydrolysed starch in to 23.13 µ moles in 30 minutes. This indicated that the specific activity of salivary amylase in starved human is 0.771 µ moles/mg/min.

From the results it is evident that, salivary amylase (72 mg) is more in the starved human saliva than the unstarved human saliva (60 mg). The hydrolysis of starch by the starved and unstarved human saliva is 400 µg and 250µg respectively. So the concentration of salivary amylase is more in the starved condition than the unstarved condition. This indicates higher enzymatic reaction in the starved human saliva than the unstarved condition.

The specific activity of starved and unstarved human salivary amylase is 1µ mole/mg-1/min-1 and 0.771µ mole/mg-1/min-1 respectively. So this indicates that, the specific activity of enzyme during starved condition is more than the unstarved condition. The starved human saliva has higher concentration and specific activity of ptyalin than the unstarved human saliva.

The production of saliva is stimulated both by the sympathetic and the parasympathetic nervous system. The saliva stimulated by sympathetic innervation is thicker, and saliva stimulated parasymp athetically is more watery. Sympathetic stimulation of saliva is to facilitate respiration, whereas parasympathetic stimulation is to facilitate digestion. Parasympathetic stimulation leads to acetylc holine (ACh) release onto the salivary acinar cells. ACh binds to muscarinic receptors and causes an increased intracellular calcium ion concentration (through the IP3/DAG second messenger system). Increased calcium causes vesicles within the cells to fuse with the apical cell membrane leading to secretion formation. Acetyl Choline also causes the salivary gland to release kallikrein, an enzyme that converts kininogen to lysyl-bradykinin. Lysyl-

bradykinin acts upon blood vessels and capillaries of the salivary gland to generate vasodilation and increased capillary permea bility. The resulting increased blood flow to the acinar allows production of more saliva. Lastly, both parasympathetic and sympathetic nervous stimulation can lead to myoepitheilium contraction which causes the expulsion of secretions from the secretory acinus into the ducts and eventually to the oral cavity. Saliva production may also be pharmacologically stimulated by so called sialagogues. It can also be suppressed by so called anti sialagogues, [8, 9,10,11,12]. Some publications revealed that starvation increased the secretion of salivary amylase[3,4],

Table1: Specific activity of saliva in starved and unstarved humans

Human saliva	Protein in 1ml saliva (µg)	Starch Hydrolysis in to Glucose (µg)	Specific activity of ptyalin (µmole/mg/min)
Starved	74	400	1.0
Unstarved	60	200	0.771

REFERENCES

1. Dodds M W., Johnson D A., Yeh C K., (2005). Health benefits of saliva: a review. J Dent; 33 (3):223-33
2. Masson P L, Carbonara A O, Heremans J F., (1966). Studies on the proteins of human saliva. Biochimica et Biophysica Acta (BBA) - General Subjects, 107(3), 485–500.
3. Yasuko Ishikawa, Cang Chen and Hajime Ishida., (1993). The effect of starvation on the diurnal variation of amylase secretion from rat parotid glands. Research in experimental medicine 19(1):255-262.
4. Brown C C., (1970). The parotid puzzle: a review of the literature on human salivation and its applications to psychophysiology. Psychophysiology, 7(1):66-85 (1970).
5. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951). "Protein measurement with the Folin phenol reagent". J. Biol. Chem., 193 (1): 265–75.
6. Miller, G.L., (1959). Use of di nitro salicylic acid reagent for determination of reducing sugar, Anal. Chem., 31, 426, 1959.
7. Stansted R M., Kneen E., and Blish M., 1939. A standardized Wohlgemuth procedure for alpha amylase activity. Cereal chem., 16: 712-723.
8. Pavlov, I.P. (1927/1960). Conditional Reflexes. New York: Dover Publications (the 1960 edition is not an unaltered republication of the 1927 translation by Oxford University Press http://psychclassics.yorku.ca/Pavlova/
9. Dorothy L. Kauffman, Anders Bennick, Max Blum, Patricia J. Keller, (1991). Basic proline-rich proteins from human parotid saliva: relationships of the covalent structures of ten proteins from a single individual. In: Biochemistry, 1991, 30 (14), 3351–3356.
10. Dawes, C (1972). "Circadian rhythms in human salivary flow rate and composition". Journal of Physiology 220: 529–545.
11. Christodoulides N, Mohanty S, Miller CS, Langub MC, Floriano PN, Dharshan P, Ali MF, Bernard B, Romanovicz D, Anslyn E, Fox PC, McDevitt JT., (2005). Application of microchip assay system for the measurement of C-reactive protein in human saliva. Lab Chip. 5(3):261-9. Epub 2005 Jan 13.
12. Tiwari M., (2011). Science behind human saliva. J. Nat Sc Biol Med; 2:53-8.