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RESEARCH ARTICLE

Ultrasonic study of adsorption in polysaccharide (starch) metabolism

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Starch metabolism due to adsorption of enzyme amylase on the starch substrate is outlined briefly. To explore the necessary conditions required for effective adsorption in biological media, ultrasonic techniques have been applied to elucidate the structural variations and component destruction in the considered systems. The ability of the enzyme amylase to break the linkages in starch (substrate) was determined from the observed ultrasonic velocity, which highlights the deciding factors of metabolism. It is concluded that the phenomenon of adsorption is decided by the surface area, the number of subunits held by the substrate, and the structure existing in the adsorbent, and above all, a relatively higher quantity of enzyme and the substrate.

Keywords acoustic parameters, molecular interaction, adsorption, starch

1 Introduction

Hundreds of reactions take place simultaneously in a living cell in a well-organized and integrated manner. The entire spectrum of chemical reactions occurring in the living system is collectively referred to as metabolism. Several methods are employed to elucidate biological, biochemical reactions and the metabolic pathways. These experimental approaches may be broadly divided into three categories, of which the present study falls into the third category as the application of physical methods. This involves either the use of isotopes or the variation in basic physical properties of the system. The application of physical properties to study biochemical change forms a major part of biophysical chemistry. Among the many interesting features in this subject is carbohydrate metabolism. The specific enzyme used by the human system for starch metabolism is amylase. It is believed that amylase is highly specific for starch and sometimes maltose, and this depends on adsorption phenomena [1]. Starch consists of amylose, ((Fig. 1) straight chain (15%–20%)) and amylopectin ((Fig. 2) branched chain (80%–85%)). The two glucose units are joined with oxygen atoms.

Ultrasound has become a powerful tool in predicting the behavior of liquid molecules in various environments, including adsorption phenomena [2,3]. The magnitude of density as well as velocity of sound in human body fluids or its constituents is of vital importance for carrying out acoustical analysis of human system or organs [4–7], as a sudden excess or reduction of sound velocity indicates abnormality [8,9]. To obtain more information on the properties of liquid mixtures, it is necessary to calculate certain acoustical parameters. A number of important and useful properties of liquid mixtures can be deduced from these parameters, which are not easily accessible by other methods (Fig. 3). If the action of amylase on Starch is to split them, then the components of medium will have large interactions, as the medium contains a greater number of starch, maltose and glucose molecules. To investigate the mechanism in the breaking of the starch by the hydrated amylase enzyme, the ultrasonic technique has been employed for the first time. The present work deals with the measurements of sound velocity, density, and viscosity in the ternary system of amylase in the aqueous solution of starch at 298.15 K. The primary aim of the present work was to elucidate the interaction phenomena between the enzyme amylase and carbohydrate (starch) molecules, thereby ascertaining the factors that favor the adsorption phenomenon in starch metabolism.

2 Experimental

All the solutions were prepared using AR grade chemicals, which were further purified by standard methods [10]. It was observed that the sound velocity increases with the increase in the percentage of amylase. However, the preparation of 7.0% amylase solution did not show thorough mixing and hence the 6.0% amylase solution was used for the entire work. The starch solutions was prepared from 1% to 6% in steps of 1% by Kim et al. [11]. The aqueous mixtures of amylase with starch were made in three different proportions as 90:10, 50:50, and 10:90 by weight, and were left for 2 h to attain complete stability. The ultrasonic velocity in the mixtures was measured using a variable path fixed frequency ultrasonic interferometer working at 2 MHz frequency (Mittal type, New Delhi, India) at 298.15 K. The accuracy of sound velocity was $\pm 0.1\ m \cdot s^{-1}$. The density and viscosity of the mixture were

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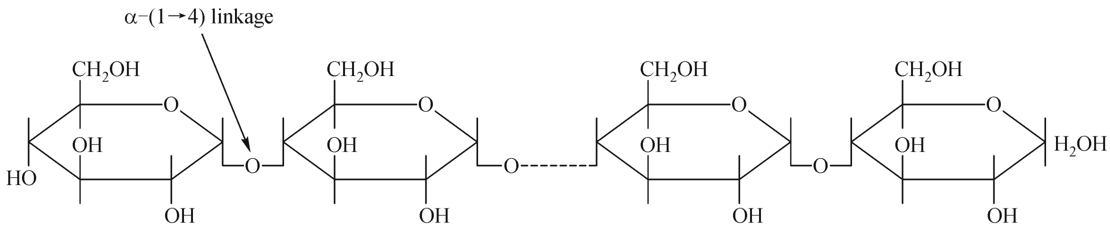

Figure 1 Structure of amylose

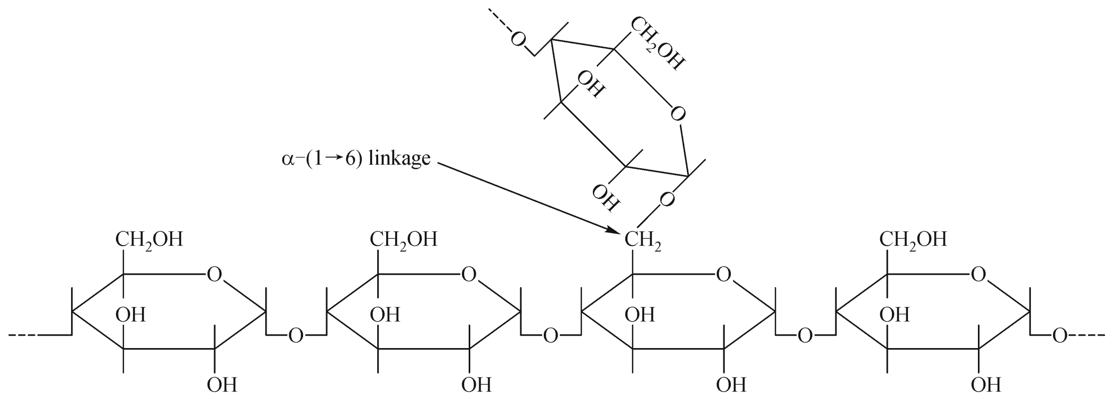

Figure 2 Structure of amylopectin

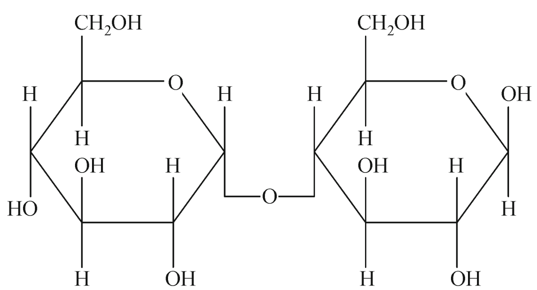

Figure 3 Structure of maltose

measured using a specific gravity bottle (5 mL) and an Oswald's viscometer (10 mL) respectively. The accuracy in density measurement was $\pm 0.0001\ \mathrm{kg \cdot m^{-3}}$ and that in viscosity measurement was $\pm 0.001\ \mathrm{mNs \cdot m^{-2}}$.

3 Theory

Using the measured parameters of sound velocity U, density ρ, and viscosity η, various thermo-acoustical parameters such as adiabatic compressibility β, free length L_f, free volume V_f, and internal pressure π_i were calculated using the following standard expressions [12]:

$$\beta = \frac{1}{U^2 \rho} \tag{1}$$

$$L_f = k_T \beta^{1/2} \tag{2}$$

$$V_f = \left(\frac{M_{eff} U}{\eta k}\right)^{3/2} \tag{3}$$

and

$$\pi_i = bRT\left(\frac{k\eta}{U}\right)^{1/2} \frac{\rho^{2/3}}{M^{7/6}} \tag{4}$$

where k_T is the temperature-dependent constant having a value 197.7×10^{-9} in M.K.S. system. k is a constant equal to 4.28×10^{8} in M.K.S. system, independent of temperature for all liquids. $M_{eff} = \Sigma x_i m_i$, where x_i is the mole fraction and m_i is the molecular weight of ith component. T is temperature; R is the universal gas constant;b is the atomic scaling factor.

4 Results and discussion

The observed values for the aqueous mixture of amylase and starch are given in Table 1. The polysaccharide (starch) taken up for the present study are hexose type glucosan [13,14]. Table 1 clearly reveals that all the measured parameters, sound velocity, density and viscosity, increase with the increase in percentage of starch. However, the variations in the viscosity are more pronounced. As the percentage rises, the number of molecules in the medium increases as well, making the

Table 1 Measured values of ultrasonic velocity U, density ρ and viscosity η for various percentage of starch in amylase (6%) solution at 298.15 K

Percentage of starch/%	U/(m·s^{-1})	ρ/(kg·m^{-3})	η /(10^3 Ns·m^{-2})
	90∶10		
1	1525.0	1018.4	0.997
2	1526.9	1019.3	1.026
3	1528.1	1020.5	1.049
4	1529.5	1021.4	1.069
5	1531.0	1022.6	1.087
6	1532.0	1023.4	1.105
	50∶50		
1	1522.4	1014.7	1.042
2	1524.7	1017.6	1.117
3	1526.6	1019.3	1.241
4	1528.7	1021.1	1.377
5	1530.0	1023.0	1.529
6	1532.5	1025.5	1.792
	10∶90		
1	1521.8	1010.5	1.084
2	1523.6	1013.7	1.151
3	1525.5	1017.0	1.209
4	1527.5	1020.0	1.265
5	1530.2	1023.3	1.763
6	1533.0	1026.6	1.888

medium denser and leading to a substantially less compressibility of the dissolved components. Further, this tends to increase the coefficient of viscosity in the mixtures. Moreover, the existing particle–particle resistance initiates some more interactions, which is highly supported by the nonlinear trend of the measured parameters.

The type of food consumed influences all the bodily fluids and their functions. Normally any type of food that humans consume majorly contains proteins, fat, and carbohydrates. The chief carbohydrate of food is starch. It consists of amylose (straight chain) and amylopectin (branched chain) consisting of glycosyl units, whose derived units are starch, maltose and glucose. The glycosyl bond is joined at 1∶4 C glucoside linkages with oxygen. The carbohydrate splitting enzymes must break down the linkages in order to form simple products [1], and the enzyme responsible for splitting starch is amylase. Starch molecules are rapidly broken down into maltose and glucose unit. As starch, maltose (Fig. 3) and glucose (Fig. 4) are reducing sugars; the course of the hydrolytic reaction is paralleled by an increase in soluble reducing materials. However, the breaking of a carbohydrate complex molecule or its subunit by hydrated amylase is found to be better than that by a pure amylase molecule, as the formation of mixture shows precipitation in the second case [15].

A keen look at Table 1 suggests that the range of density, sound velocity, and their variation with percentage of amylase is also appreciable. The nonlinear variation in these parameters with respect to the liquid composition suggests the basis of structural changes as well as interactions between like molecules and unlike molecules. In comparison in terms of proportions, in general, 90∶10 shows a relatively higher density and viscosity, whereas 10∶90 shows a relatively lower sound velocity. At all combinations, the viscosity variations are much more specific. As suggested by West et al. [14], a higher density reflects the existence of higher intermolecular interactions.

This seems to have resulted from the particle-sensitive nature of complex starch. The increase in the density of the mixture clearly reveals that more mass is accumulated in the component. This reveals the significant characteristics of the medium, i.e., though the density and velocity seem to be apparently independent of each other, it is not the case actually. For a single particle of a component in the mixture, the sound velocity depends on the size and mass of the particle. Size is important in offering cohesion effects, whereas mass is important regarding the inertial effects (Fig. 5). For the sound energy of the same strength, a reduction in velocity indicates that the inertial effects are strengthened, which is due to the accumulated mass.

The density and viscosity were observed in the order of 90∶10, 50∶50, and 10∶90. This again reflects the extent of existing intermolecular interactions. In all proportions starch

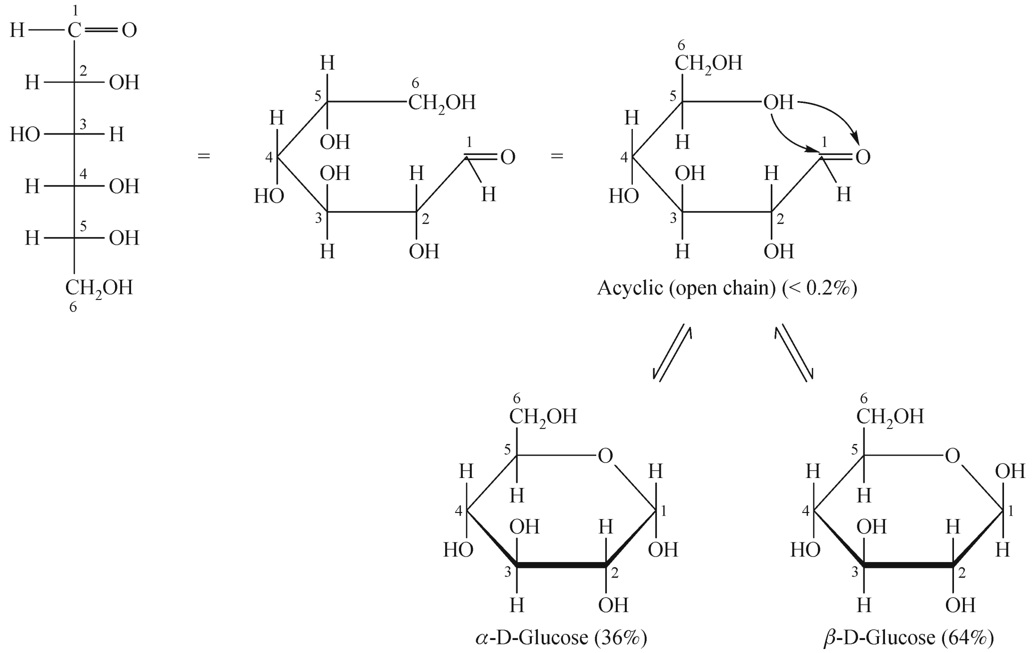

Figure 4 Structure of glucose (in aqueous medium)

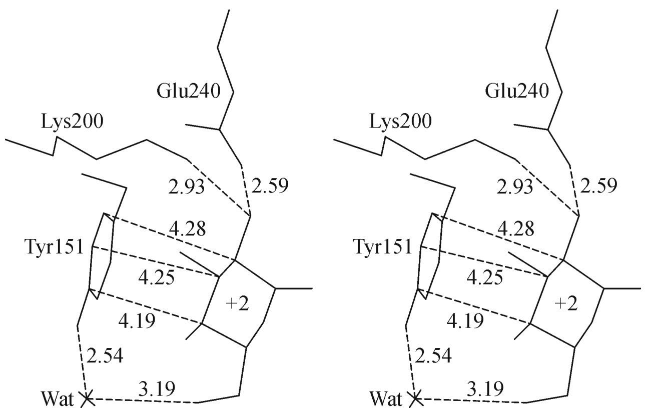

Figure 5 Structure of amylase

exist in straight and branched chain bonded with α-1, 4 glucosyl and α-1, 6 glucosyl bond, which have active groups. Such structural variations are reflected in the observed trend of the measured parameters. The metabolism of any carbohydrate molecule by the enzyme due to enzyme-substrate catalytic action leads to the availability of a bigger number of components in the medium. For the catalysis to occur in biologic system, formation of enzyme-substrate complex is a prerequisite. This happens by the adsorption of substrate on the enzyme [1]. Thus it seems that the available surface area, which is decided by the existing structure, is one of the key factors that control the catalytic actions in biological media. This was supported by the findings of Sharp [16] that the spinal structure in g-Al_2O_3 is responsible for its excellent adsorptive power.

The observed density and viscosity values reflect a reverse trend to that of velocity. As cited by Kirschner and Woods [17], polysachhcrides exhibit various conformations in the aqueous medium, which may be attributed to the gauche effect. In structural terms, the gauche effect has been defined as the tendency for a molecule to adopt the structure that has the maximum number of gauche interactions between the adjacent electron pairs and/or polar bonds [18]. This definition is demonstrative in nature and does not attempt to explain the physics behind the phenomenon. Interactions of these starch molecules with the aqueous solvent may determine the preferred conformation by disrupting intramolecular hydrogen bonding. To identify the nature and type of the interactions, some thermo-acoustical parameters have been determined and their trends are analyzed in the light of existing structural variations. From the data in Table 2, it is observed that the adiabatic compressibility in general decreases with the increase in percentage of starch for all proportions in both cases. The ease with which a medium can be compressed is given by the compressibility values. The higher compressibility value reveals that the medium is loosely packed. It is interesting to note that the higher values of β (and L_f) are observed in the order of 10∶90, 50∶50 and 90∶10 in proportion. On the basis of sound propagation in liquid, the increase in free length after mixing decreases the sound velocity. This is also in accordance with the expected decrease in compressibility following an increase in ultrasonic velocity with increasing starch concentration in the mixture. Similar results in some liquid systems were reported by Bhatti

Table 2 Calculated values of adiabatic compressibility β, free length L_f, free volume V_f and internal pressure π_i for various percentage of starch (6%) in amylase solution at 298.15 K

Percentage of starch/%	β /(10^{10} $N^{-1}\cdot m^2$)	L_f /(10^{11} m)	V_f /(10^8 $m^3\cdot mol^{-1}$)	π_i /(10^{-9} $N\cdot m^{-2}$)
		90:10		
1	4.222	4.100	1.767	2.722
2	4.208	4.093	1.698	2.759
3	4.197	4.087	1.647	2.788
4	4.185	4.082	1.606	2.813
5	4.172	4.075	1.571	2.835
6	4.163	4.071	1.536	2.857
		50:50		
1	4.252	4.114	1.604	2.837
2	4.227	4.102	1.458	2.928
3	4.209	4.094	1.256	3.072
4	4.191	4.085	1.086	3.220
5	4.176	4.077	0.936	3.380
6	4.152	4.066	0.744	3.645
		10:90		
1	4.273	4.124	1.466	2.949
2	4.250	4.113	1.360	3.016
3	4.225	4.101	1.283	3.067
4	4.202	4.090	1.217	3.113
5	4.173	4.076	0.751	3.646
6	4.145	4.062	0.689	3.745

et al. [19] and Palaniappan [20]. In comparison, in 10:90 proportions, the recorded values of compressibility are higher and in 90:10 they are lower. This shows that the presence of amylase in aqueous starch solution plays a major role for interaction. This suggests that starch shows higher interaction at all percentage of amylase. This may be attributed to the enzymatic activity in starch solution, and in the aqueous medium itself will increase the number of components as a result of the hydrolysis phenomenon. Thus, in general, interactions are stronger in higher concentration of amylase solution than in lower concentration of amylase solution. The observed similar variations in the trend of free length have confirmed this view.

From Table 2, the V_f values in all combination cases are found in general to decrease with the increase in percentage of starch, whereas the internal pressure shows an increasing trend. This suggests the loose packing of molecules inside the shield as a result of either the weakening magnitude of interactions, as suggested by Srinivasalu and Naidu [21] and Palaniappan and Nithiyanantham [22], or the availability of bigger number of sub-unit components resulting from the adsorption phenomenon in the medium.

The amount of molecules in the mixture of starch are thus predicted to be more in higher concentration, and as more amylase is available in number, maximum enzymatic activity is expected. This suggests that for the successful metabolism of a given quantity of starch, comparatively more enzyme is needed, as this can provide active sites for enzymatic action. Whereas, for a given quantity of amylase, if the carbohydrate quantity is increased, a reduction in V_f is noticed, suggesting that reduction of enzyme activity or rate of metabolism is decreased. The addition of starch or amylase increases the number of subunit components in the medium by hydrolytic and enzymatic action. These are indicated by the variation of free volume with the percentage of starch as well as with the variation of amylase. This result is further supported by the exact reverse trend shown by the internal pressure. As amylase or starch concentration is increased, it is expected that splitting will be enhanced. However, all the split components are held together by the existing adhesive forces between the components that lead to the increasing trend of internal pressure with saccharide percentage. The change of cohesive forces to adhesive forces was noticed previously by Palaniappan and Karthikeyan in some ternary systems [23,24]. This leads to the existence of specific interaction between the components of the mixtures.

5 Conclusions

The phenomenon of adsorption is decided by the surface area, the number of subunits held by the substrate, and the structure existing in the adsorbent, and above all, a relatively higher quantity of enzyme than the substrate. i) The chain length and the structure of basic unit of starch determine the strength of interactions; ii) Weak interactions are observed between unlike molecules, and strong interaction between the like molecules; iii) Among the three proportions, 10∶90 shows higher as well as lower interactions.

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