J Biochem Tech (2016) 7(2): 1058-1062
ISSN: 0974-2328

Optimization of amylase activity in the presence of various metal ions and surfactants in aqueous system

Zulfiqar Ali Raza* and Aisha Rehman

Received: 30 June 2015 / Received in revised form: 05 August 2015, Accepted: 18 August 2016, Published online: 12 September 2016

Abstract

The effect of Fe^{+2}, Mg^{+2} and Ca^{+2} cations, and sodiumdodecylsulphate (SDS) and cetyltrimethlyammonium bromide (CTAB) surfactants was investigated on amylase activity in the aqueous media. Three commercially available enzymes (Luzyme HP, Aquazym SDL and Bactosol PHC) were used for this purpose under various setups. In the first setup, individual effect of metal ions and surfactants was studied, and then based on the results from first setup; in the second setup, trend of amylase activity was studied for a concentration range of selected metal and surfactant. The third experimental setup was established to investigate the combined effect of metal ions and surfactant on amylase activity. In the case of metal ions, Fe^{2+} ions showed promoting effect on amylase activity. CTAB inhibited amylase activity whereas SDS promoted it up to a certain level. Moreover, the effect of Fe^{2+} ions and SDS concentration on activity was found that Fe^{2+} enhanced activity up to 5 mM while SDS concentration didn't show considerable effect. Combination of both Fe^{2+} and SDS showed increment in activity up to a certain level. All three commercially available amylases showed almost similar behavior towards metals and surfactants; however, the activity of Bactosol PHC was the highest than others.

Keywords*:* Amylase, Central composite design, Optimization, Surfactant

Introduction

Amylase (*EC 3.2.1*) belongs to the class of hydrolases and it hydrolyzes starch into smaller carbohydrate molecules e.g., glucose, maltose and dextrins which are water soluble. Several aspects of amylase's mode of action can be distinguished. The first aspect is hydrolysis of glucosidic bond on a molecular scale. The amylases work according to double displacement mechanism which is an acid base catalyzed reaction. They require a proton donor and nucleophile. The endo acting amylases work on this principle while exo acting amylases work via single displacement mechanism (Bijttebier et al. 2008).

Diverse applications of amylase require certain unique properties such as thermostability, ability to function efficiently at high pH, and stability in the presence of chelators and other compounds (Sarethy et al. 2012). Studies have been conducted to produce amylase from *Heliodiaptomus viduus* and *Bacillus subtilis* and then effect of pH, temperature and salt was studied (Dutta et al. 2006). Salts and surfactants can improve enzyme activity by stabilizing them against temperature and by improving wettability of fabric. Metal ions can activate enzyme activity by stabilizing the structure of enzyme-substrate complex or sensitize the substrate to the attack of enzyme or take part in ion exchange process (Athalye 2013).

Therefore, much of the effort is done to improve the amylase activity. However, combined study of enzyme activity with metals and surfactants has scarcely been reported. Present study covers effect of metal ions and ionic surfactants effect on different commercially available amylases.

Materials and Methods

Materials

Three commercially available amylases of Luzyme HP (an aqueous enzyme preparation containing alpha-amylase, BASF), Aquazym SDL (an alpha amylase from modified microorganism, Novozym), Bactosol PHC (a bacterial alpha amylase, Clariant) were donated by the respective companies. Other chemicals included: ferrous sulphate (RDH), calcium chloride (RDH), magnesium sulphate (RDH), sodium phosphate buffer (Sigma-Aldrich), 3,5-dinitro salicyclic acid (DNS, Acros), sodium potassium tartrate (Sigma-Aldrich), phenol (Sigma-Aldrich), sodium sulphate (RDH) , sodium hydroxide (Merck), maltose (Fischer Scientific), sodium

Zulfiqar Ali Raza* and Aisha Rehman

Chemistry Research Laboratory, National Textile University, Faisalabad-37610, Pakistan

* Correspondence:
Tel: +92 41 9230081, Fax: +92 41 9230098, E-mail: zarazapk@yahoo.com (ZA Raza)

Table 1. Central composite design and respective results

Enzymes	Exp. No.	Block	Fe (mM)	SDS (mM)	Design points	Activity
E1	1	I	0.30	3.00	Factorial points	0.51
(Aquazym SDL)	2	I	0.90	3.00		0.63
	3	I	0.30	8.00		0.65
	4	I	0.90	8.00		1.07
	5	I	0.18	5.50	Axial point	0.64
	6	I	1.02	5.50		0.84
	7	I	0.60	1.96		0.45
	8	I	0.60	9.04		0.85
	9	I	0.60	5.50	Center points	0.92
	10	I	0.60	5.50		0.90
E2	11	I	0.30	3.00	Factorial points	1.41
(Bactosol PHC)	12	I	0.90	3.00		1.74
	13	I	0.30	8.00		1.49
	14	I	0.90	8.00		1.76
	15	I	0.18	5.50	Axial point	1.66
	16	I	1.02	5.50		1.98
	17	I	0.60	1.96		1.21
	18	I	0.60	9.04		1.39
	19	I	0.60	5.50	Center points	1.90
	20	I	0.60	5.50		1.89
E3	21	I	0.30	3.00	Factorial points	0.51
(Luzyme HP)	22	I	0.90	3.00		0.96
	23	I	0.30	8.00		0.67
	24	I	0.90	8.00		1.02
	25	I	0.18	5.50	Axial point	0.73
	26	I	1.02	5.50		1.15
	27	I	0.60	1.96		0.58
	28	I	0.60	9.04		0.83
	29	I	0.60	5.50	Center points	1.22
	30	I	0.60	5.50		1.21

sulphite (Fischer Scientific), glucose (Sigma-Aldrich), sodium dodecylsulphate (SDS, Merck) and cetyltrimethylammonium bromide (CTAB, Fischer). All the chemicals were of analytical grade and used as received.

Design of experiment

First of all, native activities of all the three amylases were determined then the effect of metal ions (Fe^{+2}, Mg^{+2} and Ca^{+2} as 0.1, 1 mM) and surfactants (CTAB as 1 and 8 mM and SDS as 0.1 and1 mM) individually was investigated. Effect of interaction of selected metal ion and surfactant on enzyme activity was determined. The approach that was used for Table 1 is central composite approach. Value of alpha was set at by default and number of cube blocks for center points were selected 2.

Amylase assay

Amylase activity was assessed by using Miller method (Miller 1959). The standard curve (not shown) for maltose was drawn by using 0.5-5 µM/ml dilutions of DNS and taking absorbance at 540 nm. Standard factor was found by using standard curve. Enzyme activity was determined by multiplying absorbance value with the standard factor and dividing by time of incubation. One unit of amylase activity was defined as " the amount of enzyme causing release of 1 µmol of reducing sugars in one minute at 60°C for 20 minutes (Miller 1959).

Results and Discussion

Effect of metals on amylase activity

Fig.1. shows the effect of metal ions on all three commercially available amylases. It was observed that among all the three enzymes, E3 showed the highest activity. It was further observed that only Fe^{2+} enhanced amylase activity for all the test amylases. Mg^{+2} ions retarded amylase activity to greater extent on increasing concentration from 0.1 to 1.0 mM; whereas, Ca^{+2} ions showed same behavior but its retarding effect was lesser. Similar trend was observed for all the test amylases. The enhancement in amylase activity with Fe^{2+} ions could be based on its ability to interact with negatively charged amino acid residues such as aspartic and glutamic acid (Hossain and Uddin 2011). Although in most of the cases it was observed that calcium increased amylase activity but it depended on the source of amylase. Ca^{2+} ions could bind some catalytic residues and therefore inhibit amylase activity especially at higher concentration. Magnesium ion might have altered enzyme structure after binding to its sites resulting in modification in activity (Leveque et al. 2000).

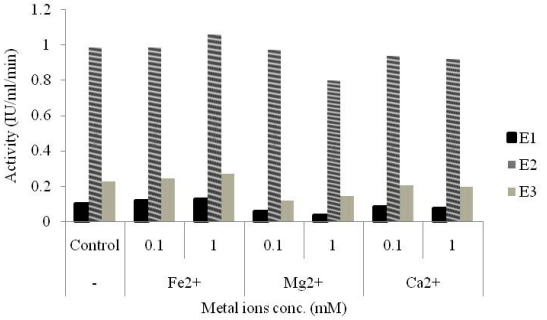

Fig. 1. Effect of various metal ions on various amylases activities

Effect of surfactant on amylase activity

Effect of ionic (SDS) and cationic (CTAB) surfactants on enzyme activity is shown in Fig. 2. It was observed that CTAB inhibited the amylase activity; whereas, SDS affected as an activator. SDS effect for both concentrations (1 and 8 mM) was much pronounced for E1 enzyme. In the case of E2 and E3 amylases, though activity increased with increasing concentration of SDS yet the effect was not much pronounced. It has also been demonstrated that the hydrolysis rates of

amylose by α-amylase from *Bacillus amyloliquefaciens* and *Bacillus licheniformis* vary in the presence of SDS; the reaction rate increases at concentrations lower than the critical micelle concentration of the surfactant and decreases over this level (Tanaka and Hoshino 2002). The EDTA inhibits enzyme activity because amylase usually contains Ca^{+2} ions as a component and surfactant has chelating properties (Prakash et al. 2011). Moreover, it could also alter enzyme structure at higher concentration (Antony et al. 2014).

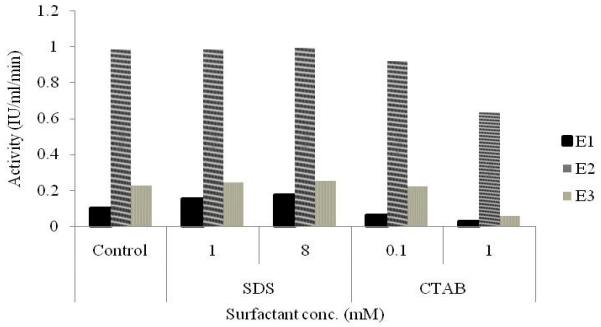

Fig. 2. Effect of various ionic surfactants on various amylases activities

Effect of Fe^{+2} concentration on amylase activity

After determining that the Fe^{+2} ions had enhanced activity of amylase, it was necessary to find the trend of activity along with increase in iron concentration. It was observed that up to a specific concentration 0.5 mM concentration of Fe^{+2} ions, amylase activity increased after that it showed a slight decrease in activity. A similar trend was observed with all the test amylases (Fig. 3).

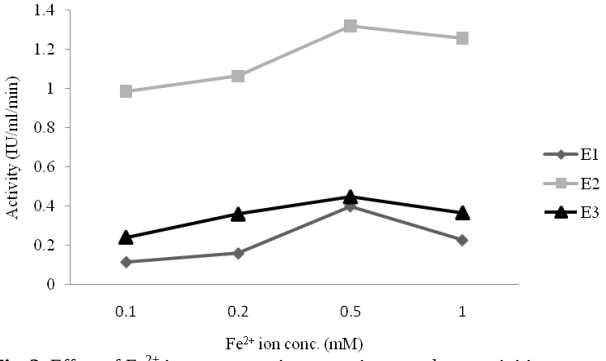

Fig. 3. Effect of Fe^{2+} ion concentration on various amylases activities

Effect of SDS concentration on amylase activity

There observed that an increase in SDS concentration didn't have appreciable effect on the amylase enzyme activity (Fig.4). It was almost same for all the concentrations of SDS; particularly, in the case of E2. Both E1 and E2 amylases showed increase in activity up to 8 mM SDS. This might be due to the fact that in starch mainly amylase and to some extent amylopectin interact with the SDS, which eventually, it gives rise to inclusion of complex molecules. Hence, the increase in activity with SDS concentration means more interaction of surfactant with the amylase (Bano et al. 2009).

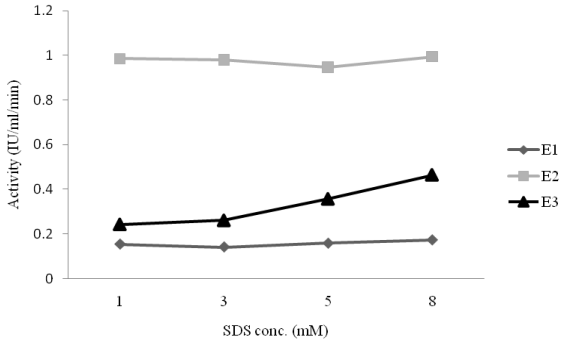

Fig. 4. Effect of SDS concentration on various amylase activities

Combined effect of Fe^{+2} and SDS on amylase activity

Central composite design was used to determine the effect of different concentrations of Fe^{+2} ions and SDS on amylase activity. Table 1 shows different experimental setups in this context. Each setup was repeated for all the three amylases. This approach helps determine the effective ranges of input variables by assigning some experiments that are out of range of the values we consider.

Analysis of variance

Table 2 shows the analysis of variance of all input variables; their interaction as well as squared terms. F-value shows the contribution of each factor for output variable and P-value shows the significance of each factor. Considering E1, it was observed that P-value of all input factors was less than 0.05 which shows that all these factors/terms have significant impact on amylase activity; while, SDS factor has the highest contribution for output variable i.e., amylase activity. Considering E2, all terms were found out to be significant except the square of Fe^{+2} ions and interaction of Fe^{+2} ions and SDS; here, the contribution of squared term of SDS is the highest. Similar was the case with E3 amylase except that squared Fe^{+2} ions term was also found out to be significant.

These different results of all amylases were due to difference in suppliers' recipes and source. However it was found that Fe^{+2} ions and SDS both have significant impact on amylase activity.

Combined effect of Fe^{+2} and SDS concentration on amylases activity

Fig. 5a shows the combined effect of Fe^{+2} and SDS on E1 activity. It was observed that by on increasing the SDS concentration, amylase activity first increased then it decreases after a specific value; and for Fe^{+2}, a linear relationship was observed. Amylase activity was the highest at 0.9 mM Fe^{+2} and 6 mM SDS. Fig. 5b shows the result of interaction of Fe^{+2} and SDS on E2 activity. A similar trend was observed as was for E1 amylase. However, a bit different results were observed in the case of E3 amylase (Fig. 5c). Although amylase activity increased on increasing SDS concentration up to a certain limit as was the case for other two enzymes. However, a parabolic behavior was also observed in case of Fe^{+2}. This behavior of amylases could be attributed to the aggregation of SDS in the baths above a certain limit of concentration this eventually inhibited the activity of Fe^{+2} ions too.

Table 2. ANOVA for amylase activity

Enzymes	Source	DF	F-Value	P-Value
E1	Fe (mM)	1	38.14	0.003
	SDS (mM)	1	72.67	0.001
	Fe (mM) × Fe (mM)	1	12.32	0.025
	SDS (mM) × SDS (mM)	1	30.78	0.005
	Fe (mM) × SDS (mM)	1	9.96	0.034
E2	Fe (mM)	1	58.36	0.002
	SDS (mM)	1	6.54	0.063
	Fe (mM) × (mM)	1	1.05	0.363
	SDS (mM) × SDS (mM)	1	159.90	0.000
	Fe (mM) × SDS (mM)	1	0.38	0.573
E3	Fe (mM)	1	164.13	0.000
	SDS (mM)	1	27.5	0.006
	Fe (mM) × (mM)	1	65.04	0.001
	SDS (mM) × SDS (mM)	1	211.31	0.000
	Fe (mM) × SDS (mM)	1	1.68	0.265

Multiple regression equation

The regression equations for each response variable in uncoded units are shown in Table 3. The high R-sq value shows the accuracy of models.

Response optimizer

Fig. 6 shows the response optimizer graphs for all three enzymes. They show the optimized values of input variables for which maximum activity of enzymes could be achieved.

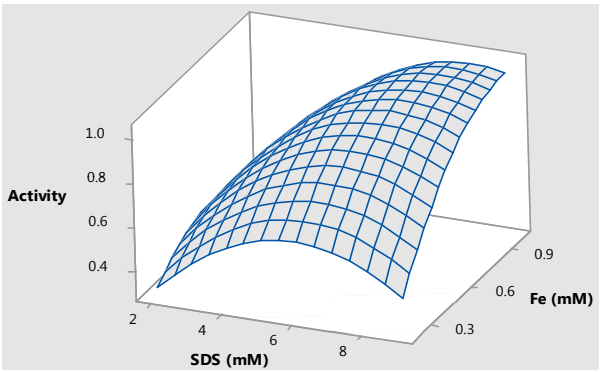

Fig. 5a

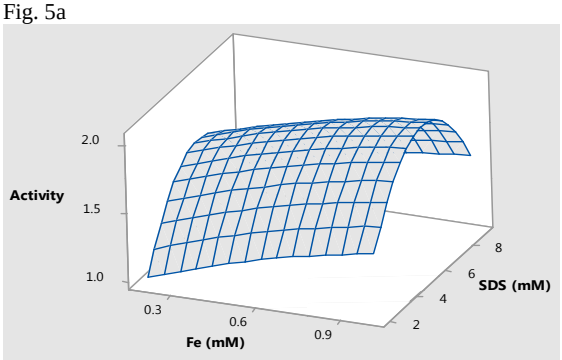

Fig. 5b

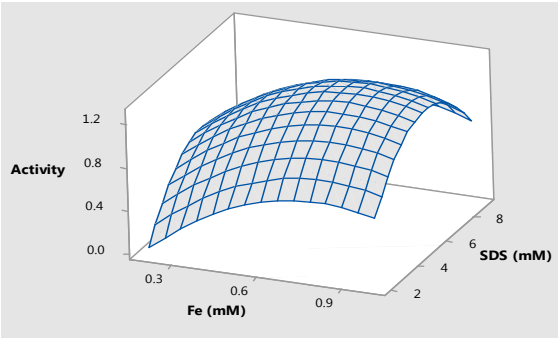

Fig. 5c

Fig. 5. Contour surface plot of combined effect of Fe^{+2} ions and SDS concentrations on activity of E1 (a), E2 (b) and E3 (c) amylases

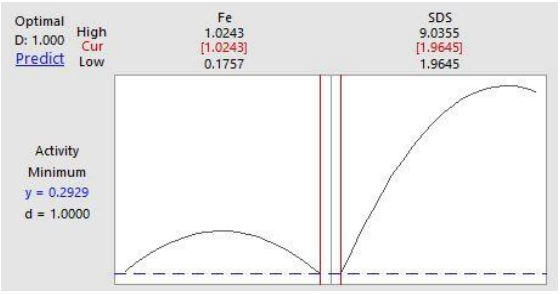

Fig. 6a

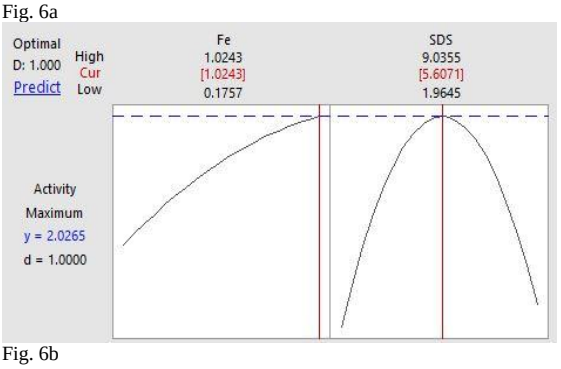

Fig. 6b

Table 3. Regression equation for each response variable in uncoded units

Response	Regression **e**quation	R-sq (%)
Activity of E1	-0.206 + 0.860 Fe + 0.2173 SDS - 0.889 Fe*Fe - 0.02000 SDS*SDS + 0.1000 Fe*SDS	97.39
Activity of E2	-0.033 + 0.915 Fe + 0.5357 SDS - 0.306 Fe*Fe - 0.04600 SDS*SDS - 0.0200 Fe*SDS	98.49
Activity of E3	-1.257 + 2.706 Fe + 0.5118 SDS - 1.618 Fe*Fe - 0.04210 SDS*SDS - 0.0333 Fe*SDS	98.43

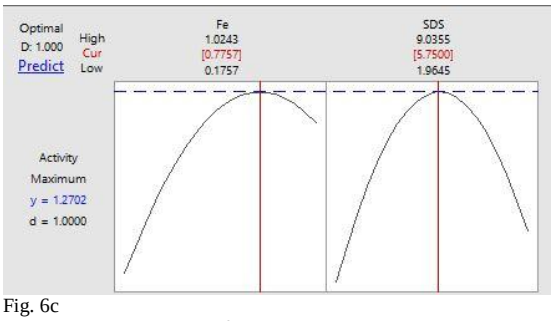

Fig. 6c

Fig. 6. Response optimizer for (a) E1, (b) E2 and (c) E3 enzyme

Conclusion

The salts and surfactants exhibited either activating or inhibiting effect on enzyme activities. As per above results, it was found that both Fe^{2+} ions and sodium dodecylsulphate showed positive effect on amylase activity. On considering the effect of two types of surfactants SDS (anionic) and CTAB (cationic) on amylase activity, it was found that the EDTA inhibited amylase activity; while, SDS promoted it up to a certain level. Moreover, the effect of Fe^{2+} and SDS concentration on activity was observed and it was found that Fe^{2+} enhanced activity up to 5 mM; while, SDS concentration didn't show considerable effect. The combination of both Fe^{2+} and SDS showed increment in amylase activity up to a certain level.

Acknowledgement

The authors acknowledge the Higher Education Commission, Islamabad for providing the research grant under NRPU.

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