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Research Article

α-AMYLASE INHIBITION AND ELECTROCHEMICAL BEHAVIOR OF SOME OXOVANADIUM (IV) COMPLEXES OF L-AMINO ACIDS

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ABSTRACT

Objective: Diabetes is complex metabolic disease having a symptom of hyperglycemia. Oxovanadium (IV) and l-amino acids are used to normalize the hyperglycemic condition. The aim of this study was to screen the α-amylase inhibitory activity of l-amino acids, their oxovanadium (IV) complexes, and electrochemical activity of oxovanadium (IV) complexes.

Methods: All the oxovanadium (IV) complexes were synthesized according to the solubility of l-amino acids; the molar ratio of metal to l-amino acid was 1:2. The synthesized oxovanadium (IV) complexes were examined for their electrochemical behavior in 0.01 M sodium perchlorate solution. Further, the oxovanadium (IV) complexes of l-amino acids and l-amino acids were screened for their α-amylase inhibitory activity using spectrophotometric assay system.

Results: The synthesized complexes were divided into four groups according to nature of amino acids. Entire complexes show simple irreversible wave for VO redox couples in −900–50 mV potential range and scan rate was 300 mV/S. All the complexes and l-amino acids were screened for their α-amylase inhibitory activity. L-Histidine and their oxovanadium (IV) complex show the minimum IC_{50} value, i.e. 4199.05 μM and 101.015 μM, respectively, in their respective groups.

Conclusion: The data obtained from our study, it reveals that the entire oxovanadium (IV) complexes are an irreversible wave for VO redox system and the l-histidine and its oxovanadium (IV) complex is the most potent inhibitor for the α-amylase. Further, the complexes show minimum IC_{50} value on comparing their respective ligands due to the interaction of Vanadyl complex to the enzyme, at the sixth vacant position of Vanadyl complex.

Keywords: Diabetes mellitus, Oxovanadium (IV) complexes, l-Amino acids, α-amylase inhibition, Cyclic voltammeter.

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INTRODUCTION

During the development of diabetes, the cellular balance of carbohydrate and lipid metabolism is affected by improper regulation. This improper regulation of carbohydrate leads to elevated post-prandial blood glucose level. Prolonged imbalance of carbohydrate interrupts the homeostasis of various physiological systems of the body, which leading to the onset of diabetes complication [1,2].

Abnormal high blood glucose level in periphery fluid can leads to a number of serious consequences, including nerve and blood vessel damage, heart disease, kidney disease, stroke, and blindness [3-5]. The manifestation of pancreatic β-cell impairment and a gradual loss of cellular responsiveness of insulin cause Type II diabetes. Since type II diabetes cases are associated with insulin insensitivity, high levels of insulin linked to obesity [6], and now oral therapeutic drugs are preferred to lower or normalize blood glucose level by physicians.

The digestion of starch is a multistep process that begins in the oral cavity with the hydrolysis of insoluble starch polymers into shorter oligomers by salivary α-amylase [7-9]. On reaching the small intestine, pancreatic α-amylase provides more extensive hydrolysis of starch. The resulting mixture then passes into the brush border of the small intestine where it is processed into glucose by the resident enzymes α-glucosidases maltase/glucoamylase and sucrase/isomaltase [8,10].

For normalizing the blood glucose level in peripheral fluids can be accomplished by oral antidiabetic drugs which control the influx of glucose into the bloodstream from the liver and the gastrointestinal track, these two strategic points for design new drugs [11]. Most therapeutic oral drugs currently in use inhibit the enzymes of gastrointestinal track. Further, most of the drugs in use are centered to inhibit the α-glucosidases since this approach also prevented the hydrolysis of common dietary sugars such as sucrose into glucose while blocking the hydrolysis of starch-derived oligosaccharides [11-13]. The α-glucosidase inhibitors miglitol, voglibose, and acarbose are iminosugar based molecules that are used in clinic practice, and unfortunately, all are associated with side effects ranging from diarrhea to hepatotoxicity [14,15]. Due to the natural consequences of displacement of di- and trisaccharides to the lower gut leads to osmotic-induced diarrhea and anaerobic fermentation [15]. α-amylase is active within the lumen of the duodenum thus, orally administered inhibitors that stay within the gastrointestinal tract will be optimally localized for amylase inhibition and will be less likely to cause undesirable side effects.

To inhibit the α-amylase, we are intended to screen the inhibitory activity of oxovanadium (IV) complexes with l-amino acids and solely l-amino acids for the sake of comparison. The agenda behind to choose oxovandium(IV), l-amino acids and their respective complexes for screening the α-amylase inhibition and electrochemical activity because we have been previously reported their antioxidant activity and found good scavenger agents[16,17].

Hence, this study sought to investigate the inhibitory effect of amino acids and their oxovanadium (IV) complexes on key enzyme linked to

diabetes (α-amylase) as well as assessing the electrochemical behavior of these complexes.

METHODS

Chemicals

Chemicals and reagents used such as porcine pancreatic α-amylase, dinitrosalicylic acid, p-nitrophenyl-α -D-glucopyranoside, sodium chloride, and sodium diphosphate were procured from SRL, India. Acarbose was sourced from Sigma-Aldrich. Sodium carbonate, methanol, potassium acetate, and starch were of analytical grade while the water was glass distilled.

Synthesis of complexes

Synthesis of oxovanadium (IV) complexes was categorized according to the solubility of amino acids.

Synthesis of complexes at 7-8 pH

A 1 mM of amino acid (Glycine, Valine, Alanine, Proline, Serine, Histidine, Arginine, Lysine, Threonine) was dissolved in 30 ml water and a transparent solution was obtained. In above solution 0.5 mM of $VOSO_4.5H_2O$ was mixed drop by drop with continuous stirring, blue/deep blue solutions were obtained. The excess solution was removed by evaporation to get the complex precipitate out on cooling.

Synthesis of complexes at 10-12 pH

A mixture of 1 mM of amino acids (methionine, asparagine, tyrosine, glutamic acid, glutamine, aspartic acid, and phenylalanine) and 1 mM of sodium acetate was dissolved in water followed by addition of 0.5 mM Vanadyl sulfate. The solution was stirred for 4 h. The excess solution was removed by evaporation to get the complex precipitate out on cooling.

Synthesis of complexes at 13-14 pH

A mixture of 1 mM of amino acids (leucine, isoleucine, and tryptophan), except cystine (0.5 mM) and 1 mole of sodium hydroxide was dissolved in water followed by addition of 0.5-mole Vanadyl sulfate. The solution was stirred for 4 h. The excess solution was removed by evaporation to get the complex precipitate out on cooling.

Cyclic voltammeter

The cyclic voltammetric measurements were carried out with a BAS instrument having an electrochemical cell with a three-electrode system. The auxiliary electrode was an $Ag/AgCl_2$. Glassy carbon was used as a working electrode, while a platinum wire electrode used as a reference electrode. The concentrations of complexes were taken 0.3 mg/ml, dissolved in supporting electrolyte 10 ml of 0.01 M solution of sodium perchlorate ($NaClO_4$) solution.

α-amylase inhibition

Pancreatic α-amylase assay was adopted from Apostolidis and Lee [18], 500 µl of different dilutions of test compounds and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing α-amylase solution (0.5 mg/mL) were incubated at 25°C for 10 min. After pre-incubation, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube. The reaction was incubated at 25°C for 10 min. The reaction was stopped with 1 mL of DNS color reagent. The closed tubes were placed in a water bath (85–90°C) for 10 min to develop color and left to cool room temperature. The reaction mixture was diluted with 10 ml of distilled water absorbance (540 nm) was read spectrophotometrically. Percentage of inhibition was expressed in terms µg/ml.

RESULTS

Synthesis of complexes

All the oxovanadium (IV) complexes were synthesized as reported earlier [16].

Electrochemical behavior of oxovanadium (IV) complexes by CV

Cyclic voltammetry is the most flexible electroanalytical technique for the study of electroactive species. The important parameters of a cyclic voltammogram are the magnitudes of the anodic peak current (ipa), cathodic peak current (ipc), anodic peak potential (Epa), and cathodic peak potential (Epc). The cyclic voltammogram of the oxovanadium (IV) complex, recorded in sodium perchlorate as supporting electrolyte. Fig. 1 voltammogram of VO+Valine shows one reduction peak Epc in cathodic direction which is assigned as follow $VO^{IV} \rightarrow VO^{III}$ at Epc=−542 mV. The oxidation peak at −360 mV is due to irreversible oxidation of VO+Valine ($VO^{III} \rightarrow VO^{IV}$). The number of electrons transferred and redox potential was obtained from the value of ΔEp= Epa-Epc and $E_{1/2=}$ (Epa+Epc)/2, respectively (Table 1) [23].

The electrochemical behaviors of all complexes have been studied by cyclic voltammetric techniques using a glassy carbon electrode in electrolyte in water under an inert atmosphere. All the complexes show simple irreversible wave for VO redox couples in −900–50 mV potential range and scan rate was 300 mV/S. The voltammogram of VO-Valine shown in Fig. 1 and the parameters of all oxovanadium (IV) complexes are represented in Table 1.

Table 1: CV parameters of oxovanadium (IV) complexes

S. No.	Complex	Epc (mV)	Epa (mV)	ΔEp (mV)	$E_{1/2}$ (mV)
1	VO+Alanine	−702	−248.3	453.7	−475.15
2	VO+Valine	−542	−360	182	−451
3	VO+Leucine	-	-	-	-
4	VO+Isoleucine	−163	−195	−32	−179
5	VO+Proline	−774	−174.7	599.3	−474.35
6	VO+Phenylalanine	−169	−220	−51	−194.5
7	VO+Methionine	-	-	-	-
8	VO+Tryptophan	−86	−143	−57	−114.5
9	VO+Glycine	−791	−161.9	629.1	−476.45
10	VO+Serine	−713.7	−187.5	526.2	−450.6
11	VO+Threonine	−752	−167.66	584.34	−459.83
12	VO+Cystine	-	-	-	-
13	VO+Tyrosine	−105	−169	−64	−137
14	VO+Glutamine	−810.4	−33	777.4	−421.7
15	VO+Asparaginine	−759.78	−231.02	528.76	−495.4
16	VO+Histidine	−742.5	−289.26	453.24	−515.88
17	VO+Arginine	-	-	-	-
18	VO+Lysine	-	-	-	-
19	VO+Glutamic acid	-	-	-	-
20	VO+Aspartic acid	−639.46	4	643.46	−317.73

α-amylase inhibition

α-amylase inhibition data (percentage inhibition at 100 and 1000 µg/ml and IC_{50} values) for oxovanadium (IV) complexes are presented in Table 2 in the same table the data of uncoordinated (free) ligands are also given for the sake of comparison. The results of inhibition of α-amylase by oxovanadium (IV) complexes at various concentrations are also presented graphically in Figs 2-9.

A scrutiny of the IC_{50} data for inhibition of α-amylase by the amino acids and their oxovanadium (IV) complexes yields the following valuable points:

1. Complexes show much higher inhibition potentials compared to the corresponding amino acids.
2. A plot of IC_{50} values for the complexes versus IC_{50} values for the corresponding amino acids yields a linear relationship with the exception of only few amino acids, namely glycine, valine, leucine, and isoleucine, Fig 10.

Figs. 2, 4, 6, and 8 represent the inhibition curve of α- amylase by oxovanadium (IV) complexes at various concentrations while Figs. 3, 5, 7, and 9 show the inhibition curve for α- amylase by l-amino acids at different concentrations. We have divided the amino acids into four groups according to nature of their side chains. Fig. 10 was plotted between the IC_{50} values of amino acid and their corresponding oxovanadium (IV) complexes, only 15 test samples except glycine, valine, leucine, and isoleucine and their respective oxovanadium (IV) complexes. The regression line was liner and the value of correlation coefficient was 0.81562, the association between IC_{50} values was strongly positive [19].

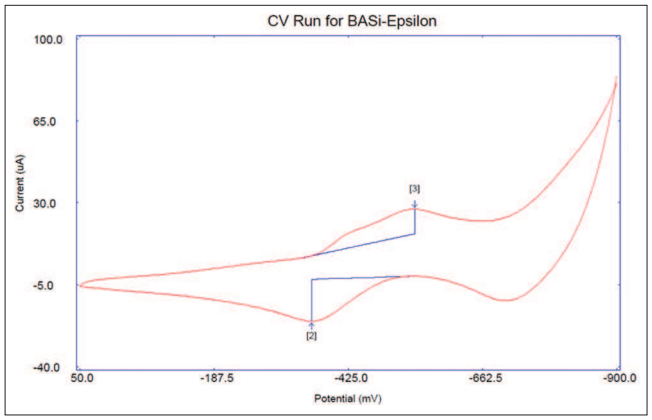

Fig. 1: Voltammogram of VO+valine

Table 2 contains the IC_{50} values in µg/ml and calculated µM, calculating the exact IC_{50} value the curve was screen for their fitted model and function to achieve the equation of line. The entire curve falls in two model categories, i.e. exponential and sigmoidal. The nature of curve explains the interaction between inhibitor and substrate (enzyme). The oxovanadium (IV) complexes of threonine, cystine, tyrosine, glutamine, asparagine, lysine, histidine, arginine, glutamic acid, and aspartic acid and these amino acid shows the sigmoid curve nature of inhibition and leftover l-amino acids and their respective complexes show exponential nature of inhibition. The sigmoid nature of inhibition pattern describes that on increasing the concentration of inhibitor they resumes or inhibits the activity of enzyme more actively while at the same concentration inhibitors showing exponential nature inhibition shows less inhibition. Moreover, on increasing the concentration of inhibitor the sigmoid nature curve shows a saturation point, it means further addition of inhibitor does not affect the activity of substrate (enzyme).

Amino acid follows the following decreasing order of inhibition potential-

Histidine (IC_{50}=4199.05 µM) >cystine (IC_{50}=4633.5 µM) >tyrosine (IC_{50}=4726.47 µM) >lysine (IC_{50}=5052.17 µM) >tryptophan (IC_{50}=5399.96 µM) >arginine (IC_{50}=5792.69 µM) >phenylalanine (IC_{50}=6190.7 µM) >methionine (IC_{50}=6193.38 µM) >glutamic acid (IC_{50}=6451.93 µM) >isoleucine (IC_{50}=6658.24 µM) >glutamine (IC_{50}=6994.71 µM) >asparagine (IC_{50}=7072.49 µM) >aspartic acid (IC_{50}=7074.07 µM) >threonine (IC_{50}=9550.65 µM) >leucine (IC_{50}=10365.4 µM) >valine (IC_{50}=11606.8 µM) >serine (IC_{50}=12272.2 µM) >proline (IC_{50}=13048 µM) >alanine (IC_{50}=15499.1 µM) >glycine (IC_{50}=25540.1 µM).

While the order of amino acids for inhibition by oxovanadium (IV) complexes are-

Histidine (IC_{50}=101.015 µM) >tyrosine (IC_{50}=125.868 µM) >Cystine (IC_{50}=135.414 µM) >tryptophan (IC_{50}=146.029 µM) >methionine (IC_{50}=146.243 µM) >arginine (IC_{50}=147.547 µM) >phenylalanine (IC_{50}=162.832 µM) >lysine (IC_{50}=164.706 µM) >glutamic acid (IC_{50}=182.575 µM) >glutamine (IC_{50}=187.02 µM) >aspartic acid (IC_{50}=202.301 µM) >threonine (IC_{50}=211.498 µM) >valine

Table 2 IC_{50} values for amino acids and their oxovanadium (IV) complexes for α-amylase inhibition

S. No.	Inhibitor	IC_{50} value		Fitted model	Function	Inhibitor	IC_{50} Value	
		µg/ml	µM				µg/ml	µM
1	VO-Alanine	257.027	1057.198	Exponential	Monomolecular growth model	Alanine	1380.813	15499.08
2	VO-Valine	65.885	220.1738	Exponential	Monomolecular growth model	Valine	1359.739	11606.82
3	VO-Leucine	88.902	271.6216	Exponential	Monomolecular growth model	Leucine	949.139	10365.44
4	VO-Isoleucine	90.007	454.3263	Exponential	Monomolecular growth model	Isoleucine	873.361	6658.237
5	VO-Proline	76.999	235.2688	Exponential	Monomolecular growth model	Proline	1502.214	13047.98
6	VO-Phenylalanine	64.371	162.8323	Exponential	Monomolecular growth model	Phenylalanine	1022.642	6190.702
7	VO-Methionine	53.139	146.243	Exponential	Monomolecular growth model	Methionine	924.114	6193.378
8	VO-Tryptophan	69.129	146.0294	Exponential	Monomolecular growth model	Tryptophan	1102.807	5399.961
9	VO-Glycine	76.988	355.3059	Exponential	Monomolecular growth model	Glycine	1937.73	25540.13
10	VO-Serine	82.012	298.0944	Exponential	Monomolecular growth model	Serine	1289.683	12272.18
11	VO-Threonine	64.122	211.4975	Sigmoidal	Logistic	Threonine	1137.673	9550.646
12	VO-Cystine	73.874	135.4142	Sigmoidal	Logistic	Cystine	1113.429	4633.496
13	VO-Tyrosine	53.786	125.8679	Sigmoidal	Logistic	Tyrosine	856.389	4726.469
14	VO-Glutamine	66.811	187.0195	Sigmoidal	Logistic	Glutamine	1022.277	6994.711
15	VO-Asparagine	64.217	175.8306	Sigmoidal	Logistic	Asparagine	1061.863	7072.486
16	VO-Histidine	37.907	101.0151	Sigmoidal	DoseResponse	Histidine	651.525	4199.053
17	VO-Lysine	64.789	164.7063	Sigmoidal	DoseResponse	Lysine	829.616	5052.165
18	VO-Arginine	60.987	147.5465	Sigmoidal	DoseResponse	Arginine	1009.087	5792.692
19	VO-Aspartic Acid	66.99	202.3006	Sigmoidal	Logistic	Aspartic Acid	941.558	7074.065
20	VO-Glutamic Acid	65.581	182.5747	Sigmoidal	Logistic	Glutamic Acid	949.272	6451.927

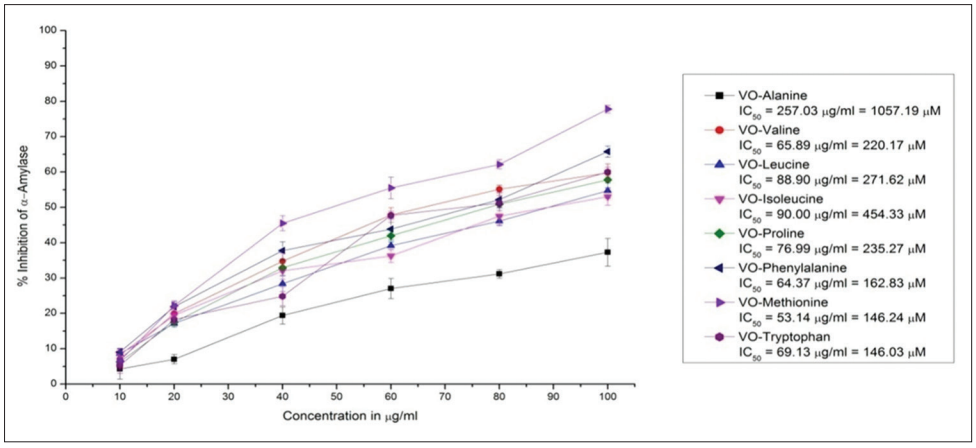

Fig. 2: Inhibition curve of oxovanadium (IV) complexes of Group A

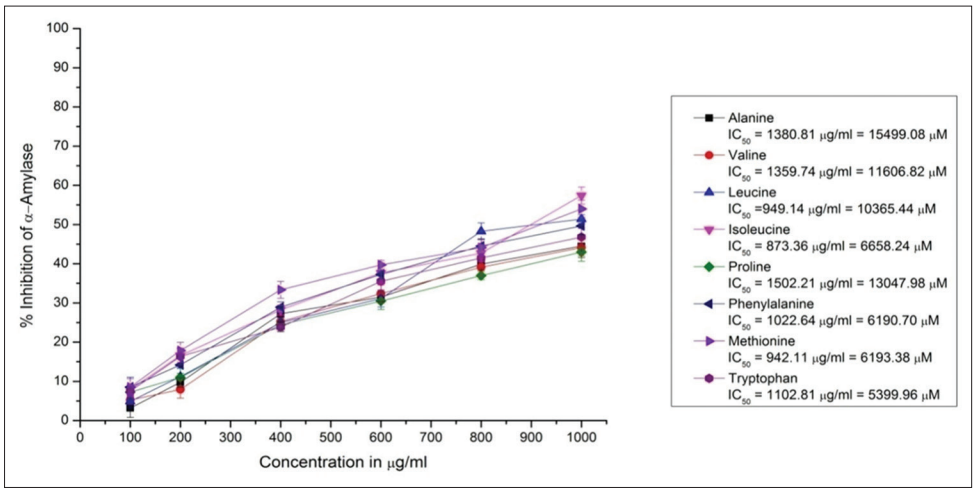

Fig. 3: Inhibition curve of amino acids of Group A

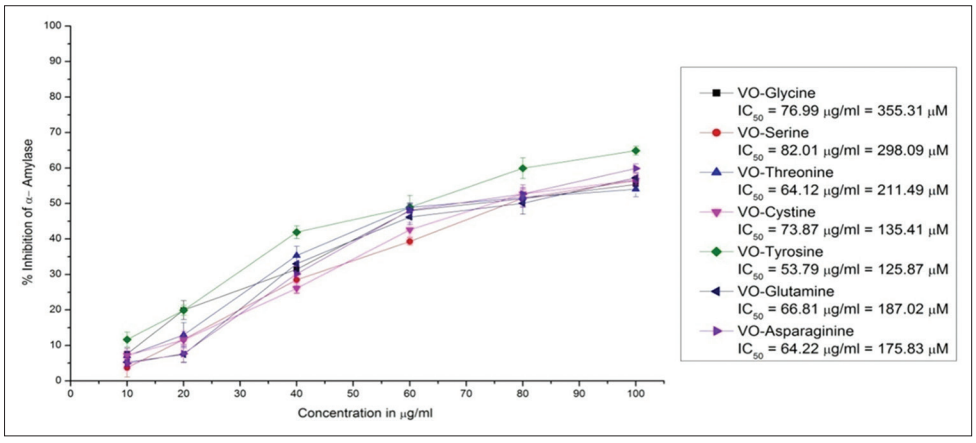

Fig. 4: Inhibition curve of oxovanadium (IV) complexes of Group B

(IC_{50}=220.174 μM) >proline (IC_{50}=235.269 μM) >leucine (IC_{50}=271.622 μM) >serine (IC_{50}=298.094 μM) >glycine (IC_{50}=355.306 μM) >isoleucine (IC_{50}=454.326 μM) >alanine (IC_{50}=1057.2 μM).

DISCUSSION

Before the discovery of insulin in 1922 by Banting and Best French physicians Lyonnet *et al.* found that sodium metavanadate ($NaVO_3$) improved the state of human diabetic patients [20]. The modern era of studying the antidiabetic properties of vanadium was initiated in 1985 by John McNeill, who monitored the cardiac function of rats with streptozotocin -induced diabetes after treatment with Vanadyl sulfate, since then there are numerous biological activities have been studied to find the impact of inorganic and organic vanadium derivatives in induced diabetes animal models and *in vitro* assay system by various workers [21-24].

Amino acids are the building blocks of proteins found in structural tissues of the body. Amino acids are essential to life in free or polymeric

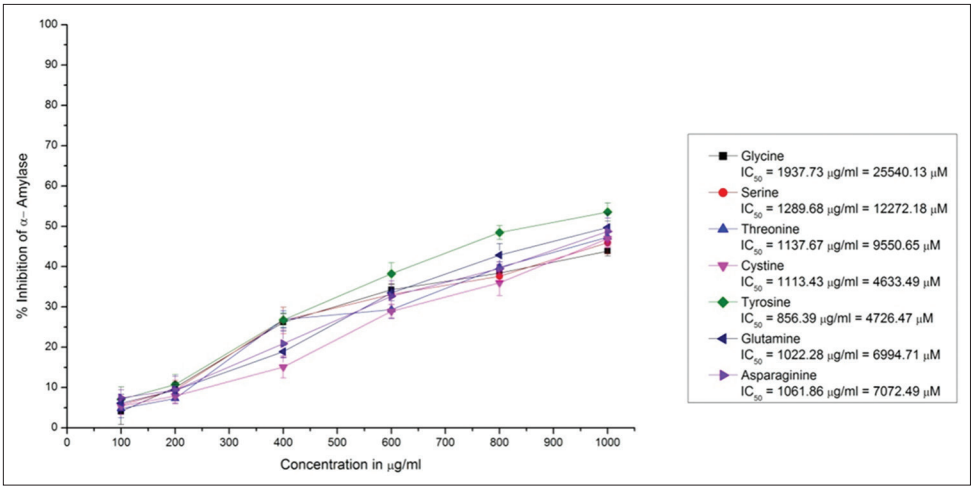

Fig. 5: Inhibition curve of amino acids of Group B

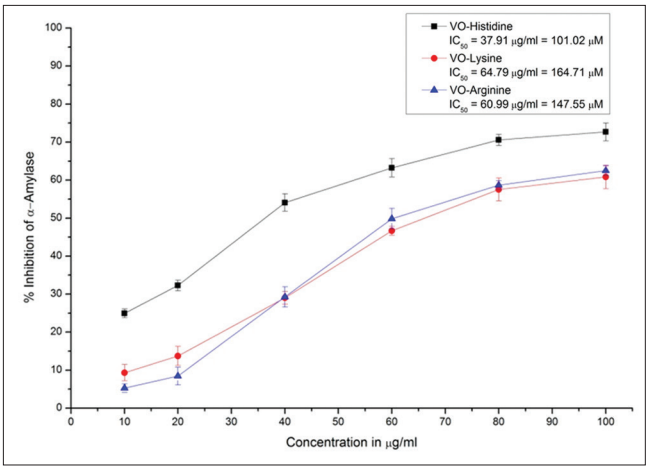

Fig. 6: Inhibition curve of oxovanadium (IV) complexes of Group C

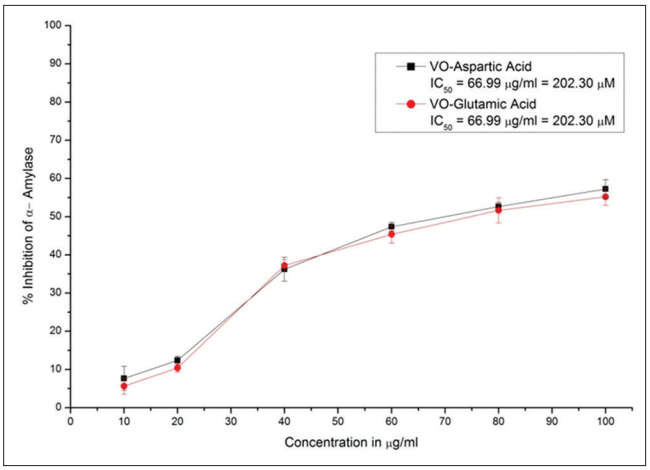

Fig. 8: Inhibition curve of oxovanadium (IV) complexes of Group D

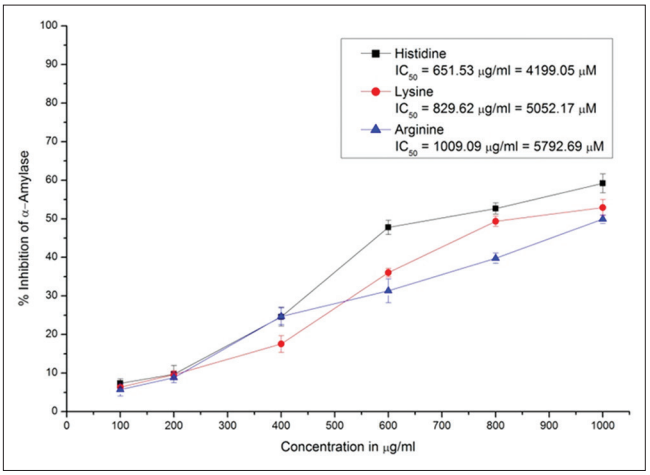

Fig. 7: Inhibition curve of amino acids of Group C

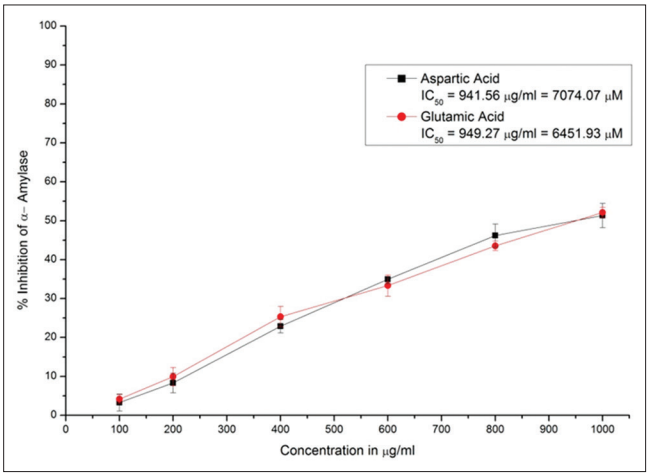

Fig. 9: Inhibition curve of amino acids of Group D

form as peptides. Amino acids play important roles in activities, such as neurotransmission, pH regulation, cholesterol metabolism, pain control, detoxification, and control of inflammatory response [25]. Some metabolic steps of amino acids related to vascular complications (methionine and arginine) exhibit a defective response to insulin in type-2 DM with nephropathy [26,27]. Obesity and insulin resistance are known to induce by proinflammatory in type 2 diabetes, together with the cause adverse effects of hyperglycemia and hyperlipidemia and leads to the progressive dysfunction and demise of pancreatic β-cells. There are many workers reported that amino acids are also responsible for secretion of insulin and metabolism of glucose [27-30].

Therefore, we aimed to screen the inhibitory effect of l-amino acids and their respective oxovanadium (IV) complexes on α-amylase.

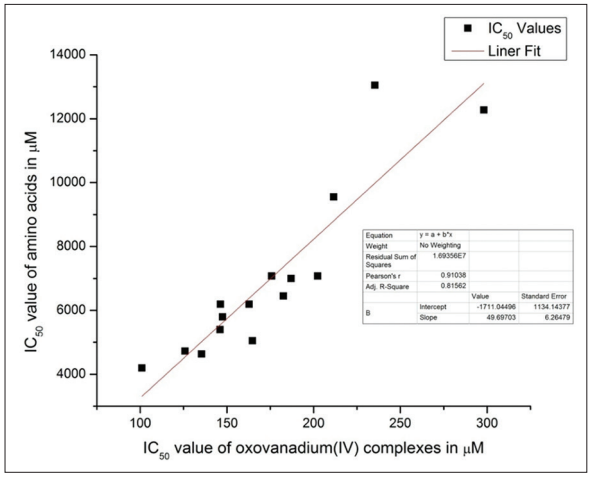

Fig. 10: Correlation plot of IC50 values (μM) of amino acids and their oxovanadium (IV) complexes for α-amylase

For the characterization of inhibitor (oxovanadium [IV] complexes), we have used cyclic voltammeter. The voltammetric method is satisfy many of the requirements for this analysis particularly because of voltammeter provide an idea about the state of oxidation of metal, rapid response, high sensitivity, low cost, simplicity, and relatively short analysis time. Misra *et al.* also reported that oxovanadium (IV) complexes have a vacant six coordinates which interact to the enzyme to inhibit their activity [34].

All the ligands (l-amino acids) of the present study fall into the category of the de melo Borges *et al.* [31] and contain phenyl, hydroxyl, imido, and carbonyl groups. Studies on structure–activity relationship on such compounds have shown that this carboxyl, hydroxyl, and amino groups are fundamental for their inhibition activity [32]. Madeswaran *et al.* studied computationally, interaction of the ligand/complex models generated after successful docking with α-amylase and concluded that the parameters such as hydrogen bond interactions, π – π interactions, binding energy, and play critical role by binding with active site residues with enzyme [33].

Oxovanadium (IV) complexes show much stronger α-amylase inhibition compared to the corresponding ligands because, oxovanadium (IV) complex are of five coordinated. The sixth coordination of oxovanadium complex is vacant which bind to the enzyme [34,35]. Cornman *et al.* [35] have earlier suggested formation of such a bond between the metal ion and protein side chain for inhibition or activation of the enzymes. The inhibitors can be further stabilized in the active site through hydrogen bonds with catalytic residues and the establishment of hydrophobic contacts in a cooperative fashion. Further, workers suggest that α-amylase can be inhibit, if inhibitor interact with 8 amino acids, with the enzyme binding site, which are Tyr-Gln-Ser-TrpArg-Tyr-Ser-Gln [36-38].

CONCLUSION

The data obtained from cyclic voltammeter its appears all the oxovanadium(IV) complexes of l-amino acids are irreversible waves. The l-amino acids and their oxovanadium(IV) interact with α-amylase to inhibit its catalytic activity. VO-Histidine and l-Histidine are most promising agents for inhibiting α-amylase among all complexes and l-amino acids.

AUTHOR'S CONTRIBUTION

All the authors contributed equally to planning, conductance of study, interpretation of results and writing.

CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

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