Ionic liquids as a strategy to enhance the catalytic performance of commercial proteases

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Abstract

This study evaluated the effects of different ionic liquids (IL), namely choline chloride, tetramethylammonium bromide and 1-ethyl-3-methylimidazolium bromide, on the enzymatic activity of the commercial proteases Alcalase® 2.4L, Flavourzyme® 500L and Neutrase® 0.8L. The results showed that the most adequate combinations of enzyme/IL were: Neutrase® 0.8L/choline chloride (0.5 mM); Flavourzyme® 500L/choline chloride (5 mM) and Alcalase® 2.4L/tetramethylammonium bromide (2000 mM), with which relative increases of 20, 15 and 150%, respectively, were detected in their activities.

Key words: ionic liquids, proteases, enzymatic activity

Introduction

The term ionic liquid (IL) can be used to describe asymmetric organic salts composed entirely of ions and which have low melting points. These compounds have been highlighted due to their application in ecologically cleaner catalytic processes. In addition, recent studies have indicated the ability of IL to modify protein structures, positively affecting the catalytic activity of enzymes.

Thus the objective of this work was to evaluate the effect of the ionic liquids choline chloride, tetramethylammonium bromide and 1-ethyl-3-methylimidazolium bromide on the enzymatic activity of the commercial proteases Alcalase® 2.4L, Flavourzyme® 500L and Neutrase® 0.8L.

Results and Discussion

The results obtained (Table 1) showed that the enzyme activity was directly affected by the different protease/IL combinations and also by the concentration. In general, an initial increase in the relative enzyme activities was observed with the increase in IL concentration, followed by a subsequent decrease.

The combination of Neutrase® 0.8L and choline chloride resulted in increases of up to 22% in the initial protease activity. The correlation analysis showed a positive correlation between the IL concentration and the relative enzyme activity in the range from 0 to 0.5 mM (Pearson coefficient = 0.91 and p -value = 0.09). On the other hand, negative correlation was detected between the relative enzyme activity and IL concentrations ranging from 0.5 to 500 mM (Pearson coefficient = -0.73 and p -value = 0.06).

For the combination Flavourzyme® 500L and choline chloride, the correlation analysis indicated a positive correlation between the enzyme activity and IL concentration ranging from 0 to 50 mM (Pearson coefficient = 0.73 and p -value = 0.04).

A positive correlation was observed between the relative enzyme activity of Alcalase® 2.4L and concentrations of tetramethylammonium bromide ranging from 0 to 2000 mM (Pearson coefficient = 0.98 and p -value < 0.001).

Table 1: Relative activity (%) of the commercial proteases at different concentrations of the ionic liquids

[] (mM)	Relative Activity (%)		
	IL 1	IL 2	IL 3
Neutrase® 0.8L			
0.0	100.0 ^b	100.0 ^{a,b,c,d}	100.0 ^a
0.25	101.59 ^b	108.20 ^a	102.08 ^a
0.50	122.22 ^a	105.65 ^{a,b}	105.22 ^a
5.0	105.42 ^b	94.41 ^{b,c,d,e}	109.62 ^a
25.0	74.24 ^c	87.01 ^{d,e}	84.68 ^b
50.0	57.73 ^d	86.58 ^e	71.41 ^c
Flavourzyme® 500L			
0.0	100.0 ^{b,c}	100.0 ^{a,b}	100.0 ^{b,c}
0.5	96.78 ^{b,c}	92.77 ^{b,c}	109.52 ^a
5.0	114.32 ^a	108.39 ^a	103.54 ^{a,b}
50.0	115.31 ^a	108.83 ^a	100.52 ^{b,c}
250	99.48 ^{b,c}	93.45 ^{b,c}	70.75 ^d
500	91.18 ^c	82.38 ^{d,e}	38.22 ^e
Alcalase® 2.4L			
0.0	100.0 ^a	100.0 ^a	100.0 ^a
0.05	104.51 ^a	104.81 ^a	107.40 ^{b,c}
2.50	104.45 ^a	113.43 ^a	109.96 ^{b,c}
250	105.57 ^a	142.75 ^b	77.33 ^d
500	130.52 ^b	139.51 ^b	20.53 ^e
2000	Not done	244.40 ^c	Not done

[] = concentration; IL 1 = Choline chloride; IL 2 = Tetramethylammonium bromide; IL 3 = 1-ethyl-3-methylimidazolium bromide.

Conclusions

The results obtained showed that the ionic liquids choline chloride, tetramethylammonium bromide and 1-ethyl-3-methylimidazolium bromide had a positive effect on the catalytic activity of the proteases Alcalase® 2.4L, Flavourzyme® 500L and Neutrase® 0.8L. The most prominent and positive effect was detected for the combination Alcalase® 2.4L with the ionic liquid tetramethylammonium bromide (2000 mM), with which an increase of approximately 250% in the enzymatic activity was observed.