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Bioseparation of alpha-amylase by forming insoluble complexes with polyacrylate from a culture of *Aspergillus oryzae* grown in agricultural wastes

María Cecilia Porfiri, Beatriz M. Farruggia, Diana Romanini *

Laboratory of Chemical-Physical Applied to Bioseparation, Facultad de Cs, Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario – CONICET, Suipacha 570, 2000 Rosario, Argentina

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ABSTRACT

Precipitation of insoluble complexes between alpha-amylase from *Aspergillus oryzae* and polyacrylic acid was studied by us in a previous work as a strategy of protein concentration and purification. Here we studied the effect of these polyelectrolytes on the stability of the enzyme at the pH of higher interaction (3.00), as well as the stability of the precipitates formed after applied the above methodology. The polymers showed a stabilizing effect on the activity of alpha-amylase at pH 3.00, given the kinetic constants achieved when adjusting the experimental data to a process of first order inactivation: $2.20e^{-2} \pm 2e^{-3}$ for alpha-amylase alone, $7.7e^{-3} \pm 3e^{-4}$ and $8.2e^{-3} \pm 2e^{-4}$ for polyacrylic acid 240,000 and 100,000, respectively.

Also, this stabilizing effect is intensified when complexes are in the solid precipitated form before redissolve, given the lower kinetic constants of inactivation obtained: $1.7e^{-3} \pm 2e^{-4}$ and $2.6e^{-3} \pm 2e^{-4}$ for polyacrylic acid 240,000 and 100,000, respectively.

In a subsequent step, a strain of *A. oryzae* was used as enzymatic source. In order to stimulate alpha-amylase production and secretion by the microorganism, we used a minimum culture medium with wheat processing residues as substrate. This has economic and environmental benefits, because of the recycling of agricultural wastes. This culture showed a maximum productivity at the fourth day of incubation.

When the technique of concentration and purification of alpha-amylase was implemented from this culture we obtained purification factors around two and recoveries around 70%. These results demonstrate that this methodology is suitable for the concentration and purification of alpha-amylase from *A. oryzae*. Besides, it is inexpensive since it uses agricultural materials without commercial value.

1. Introduction

Affinity precipitation has been subject of study for many years, but has received more attention in the last time due to the development of new materials for its implementation [1]. Both natural and synthetic polyelectrolytes can strongly interact with proteins of opposite charge and form insoluble complexes that are the bases for protein concentration and separation from a heterogeneous mixture [2–5]. Several techniques have been used to characterize polymer–protein complexes and have provided information about different aspects of them. These systems can be followed by turbidimetric techniques since these complexes can significantly increase the turbidity of the medium [6,7].

We have previously demonstrated that precipitation of insoluble complexes between purified alpha-amylase (α-Amy) and polyacrylic acid (PAA) can be used as a strategy of alpha-amylase concentration and purification [8]. We also concluded that the enzyme forms insoluble complexes with polyacrylic acid at pH under 5.00 (PAA 100,000 Da) and 4.00 (PAA 240,000 Da) with a molar ratio PAA/alpha-amylase 1/52 and 1/154, respectively [8]. Besides, we have previously designed a methodology to purify alpha-amylase by precipitating the enzyme with excess of polyelectrolyte at pH 3.00 and redissolving it at pH 6.00 (around mayor stability of the enzyme) with high tendency of the enzyme to precipitate (recoveries: 73.79% and 64.26% for PAA 240,000 Da and 100,000 Da, respectively) [8].

So that it is fully functional it is necessary that the enzyme is stable after applying the conditions of the designed methodology. This aspect was studied in the present paper by following the kinetics of inactivation of α-Amy at the pH of precipitation (3.00) in the presence of the polyelectrolytes and in the precipitated form.

Alpha-amylases are members of family 13 in the classification of glycoside hydrolases (according to Henrissat [9]). *Aspergillus*

* Corresponding author. Address: Laboratorio de Fisicoquímica Aplicada a Bioseparación, Facultad de Cs, Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 570, S2002RLK Rosario, Argentina. Tel.: +54 341 4804592 253; fax: +54 0341 480 4598.
E-mail address: dromanini@conicet.gov.ar (D. Romanini).

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oryzae has received increased attention as a favorable host for the production of heterologous proteins because of its ability to secrete a variety of high-value proteins and industrial enzymes, e.g., alpha-amylase [10–12] The enzyme synthesized from *A. oryzae* is an extracellular endo-acting hydrolase that gives large oligosaccharides as products of starch degradation because of scission of internal alpha-1,4-linkages and its three-dimensional structure has been well investigated by X-ray crystallography [13].

Starch is a polysaccharide that is widely distributed in nature as a reserve of stored energy in many species of plants, and occurs extensively in waste materials produced from the processing of plant raw materials. Starch-processing waste is produced in large quantities and causes pollution problems [14].

The aims of this work were: 1 – to analyze the effect of the polyelectrolytes on the kinetics of inactivation of alpha-amylase at pH 3.00; 2 – to isolate the enzyme with PAA from a culture of *A. oryzae* grown at the expenses of residues of wheat processing as starch source.

2. Experimental

2.1. Chemical

Alpha-amylase (α-Amy) from *A. oryzae* was purchased from Sigma Chem. Co. (USA) and polyacrylic acid, sodium salt (PAA), sol. in water, molecular average mass 240 kDa, 25% (W/W) (PAA 240,000) and 100 kDa, 35% (W/W) (PAA 100,000) were purchased from Aldrich and used without further purification. Phosphate buffer solutions of different pH were prepared at concentration of 50 mM and were adjusted with NaOH or HCl.

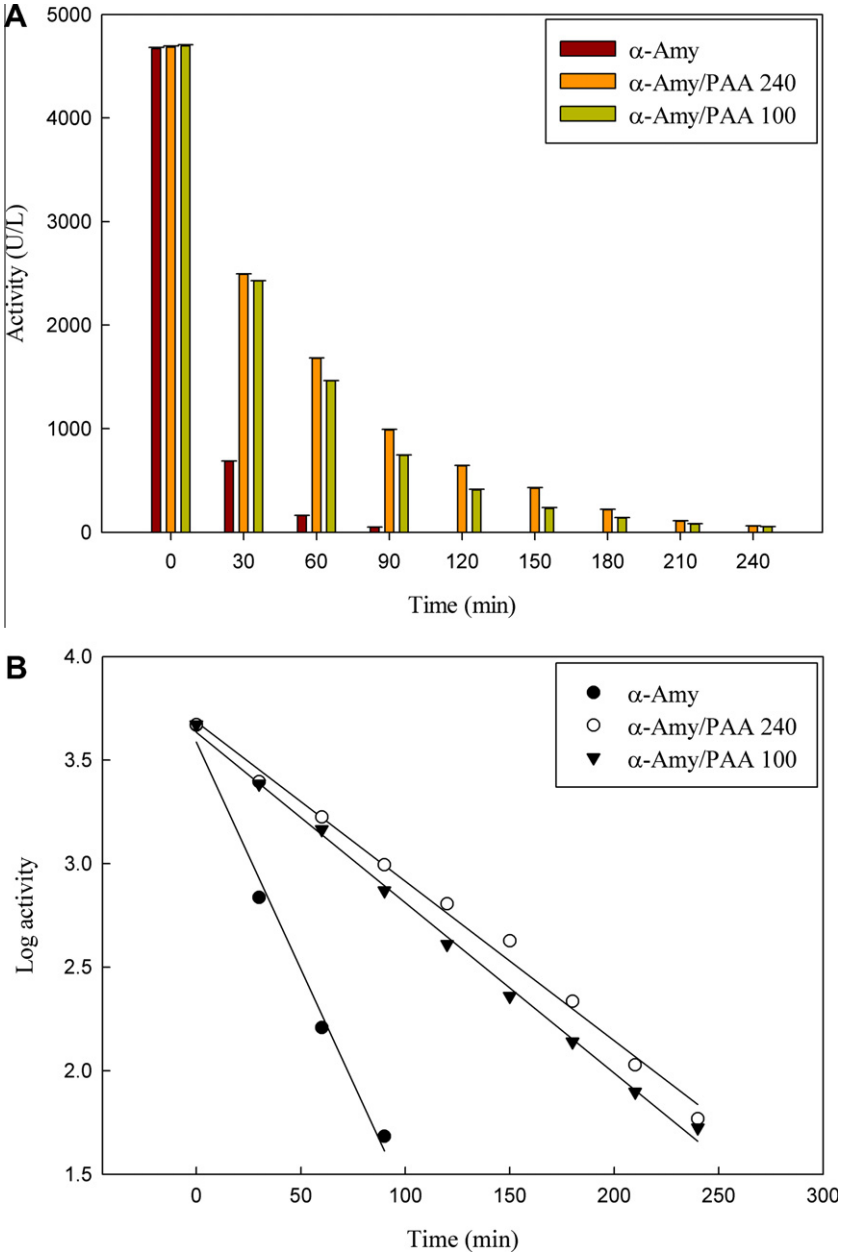

Fig. 1. A – Stability (activity vs. time) of alpha-amylase 2.75 mg/ml at pH 3.00 in the absence (—) and presence of polyacrylic acid 240,000 (—) and 100,000 (—) in a polymer:protein ratio 1:88 and 1:25, respectively. B – First-order kinetics of inactivation (log activity vs. time) of alpha-amylase 2.75 mg/ml at pH 3.00 in the absence (●) and presence of polyacrylic acid 240,000 (○) and 100,000 (▼) in a polymer:protein ratio 1:88 and 1:25, respectively.