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Stabilization of Alliinase from Garlic by Osmolytes and the Mannose-Specific Lectin ASAI

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Abstract: Objectives: Alliinase is a pyridoxal-5'-phosphate (PLP)-dependent enzyme responsible for the production of diallyl thiosulfinate (allicin), the biologically active component of garlic, from alliin. The use of allicin for treatment of various diseases has been proposed but it is very unstable in the blood stream. This difficulty can be overcome by administration of alliin, together with a conjugate of alliinase directed towards the target cells. This, in turn requires a stable and active form of the enzyme. In this study we evaluate the stability of alliinase itself, in the presence and absence of osmolytes, as well as that of its catalytically active complex with a mannose-specific lectin, ASAI (*Allium sativum* agglutinin I), also presents in garlic. Methods: Alliinase, and ASAI were both purified from garlic cloves. Thermal stability of alliinase itself, and of its complexes with PLP and ASAI, in the presence and absence of osmolytes, was analyzed by monitoring enzymic activity, and using DSC (differential scanning calorimetry). Key findings: PLP exerts only a minor influence on alliinase structure and stablity. But both osmolytes and ASAI stabilize the enzyme considerably. Conclusions: The principle finding is that ASAI greatly stabilizes alliinase. Thus, the lectin-enzyme complex, which can be lyophilized and stored until used, provides an effective formulation of alliinase for generation of allicin from alliin *in vivo*.

Key words: Alliinase, osmolyte, mannose-specific lectin ASAI, pyridoxal 5'-phosphate.

Abbreviations

PLP	pyridoxal 5'-phosphate;
DSC	differential scanning calorimetry;
ASAI	*Allium sativum* agglutinin I;
Al-ASAI	alliinase-ASAI complex; CD, circular dichroism;
TMAO	trimethylamine *N*-oxide.

1. Introduction

Allinase (Cys sulfoxide lyase, alliin lyase, C-S lyase; EC 4.4.1.4) is a pyridoxal-5'-phosphate (PLP)-dependent enzyme found in plants belonging to the genus *Allium* (that includes garlic, onions, shallots and leeks), and is ultimately responsible for the generation of volatile and odorous sulfur-containing compounds. Alliinase is especially abundant in garlic, accounting for 10% of the soluble proteins in garlic cloves [1]. The enzyme catalyzes α,β-elimination of the non-protein amino acid alliin, (+)-*S*-allylcysteine sulfoxide, to produce allicin (diallyl thiosulfinate), pyruvate, and ammonia, as shown in Fig. 1.

Alliinase is a homodimer in which each subunit contains 448 amino-acid residues, and has a molecular mass of 51,500 [2, 3]. It has been crystallized, and its three-dimensional structure has been solved [4-7]. The product of the action of the enzyme on alliin, allicin, is the biologically active component of garlic that is responsible for its pungent odor, and for a variety of biological and therapeutic effects including prominent antibiotic effects, decrease of blood pressure as well as antiatherosclerotic and antitumor activities [8, 9]. Alliinase is a mannose-rich glycoprotein with four potential *N*-linked glycosylation sites per monomer

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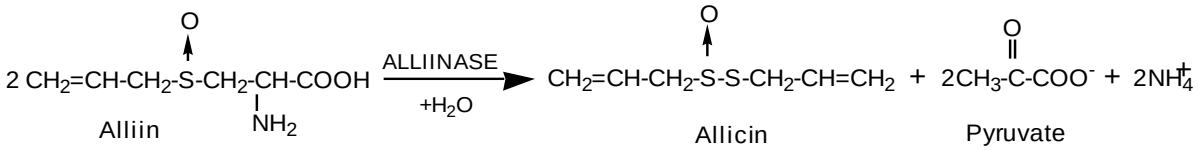

Fig. 1 The reaction of alliin catalyzed by alliinase.

[3, 7]. Interestingly, garlic cloves also contain two mannose-specific lectins, *Allium sativum* agglutinins I and II, (ASAI and ASAII) [10]. ASAI has been shown to recognize monosaccharides in a mannosyl configuration, as well as high-mannose oligosaccharide chains and glycoproteins with high mannose content [11]. Alliinase was earlier shown to form a complex with ASAI [12, 13].

Allicin is an unstable compound that disappears from the blood within minutes after injection [14]. Hence, the most reliable way to ensure effective delivery of allicin is *in-situ* generation from its precursor alliin by use of a targeted derivative of alliinase. Targeting is achieved by conjugating the enzyme to site-specific antibodies. Such conjugates produced pronounced therapeutic effects in experimental animals bearing various types of tumor [15-17] or suffering from pulmonary aspergillosis [18] when injected prior to administration of alliin.

Pure garlic alliinase is known to be thermolabile, irreversibly inactivated by denaturants, and rather unstable upon storage [19, 20]. Since alliinase is significantly stabilized by glycerol, it is usually studied in the presence of 10% glycerol [3, 19-22]. Sucrose has also been found to preserve alliinase activity [23]. Both compounds are osmolytes, and are also known as chemical chaperones, due to their capacity to stabilize proteins [24-26]. Other chemical chaperones also include methylammonium derivatives such as trimethylamine *N*-oxide (TMAO), polyols, and several amino acids and their derivatives [27, 28]. One explanation offered for their stabilizing effect is that they act by replacing bound water, thus shifting the equilibrium from an unfolded to a folded state of the protein [24-26, 29]. However, the mechanism, by which osmolytes affect protein stability, is still actively studied and debated [30, 31].

DSC (differential scanning calorimetry) provides direct information concerning the thermodynamic characteristics of the process of thermal denaturation of proteins [32-35]. Several attempts have been made to utilize DSC to study the influence of osmolytes on protein stability [30, 31, 36, 37]. In the present study, the effects of several widely used osmolytes on the irreversible denaturation of alliinase were analyzed using DSC. We also used DSC to analyze similarly the thermodynamic characteristics of the complex of alliinase with ASAI.

2. Materials and Methods

2.1 Chemicals

5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), pyridoxal 5'-phosphate (PLP), chymotrypsin, guanidine-HCl, HEPES, PEG-8000, D-(+) Mannose, methyl-D-mannoside, divinyl sulfone, D-sorbitol, and trimethylamine N-oxide (TMAO) were purchased from Sigma-Aldrich (St-Louis, MO). CL-Sepharose 6B, ConA-Sepharose, Phenyl-Sepharose 6 Fast Flow (high sub), HiLoad 26/60 Superdex-200, and HiPrep 26/10 desalting columns were purchased from GE-Pharmacia (Amersham, UK). Protein was concentrated with YM-30 centrifugal filter device (Amicon, Millipore, Beverly, MA). 2-nitro-5-thiobenzoate (NTB) was prepared as described [38]. The mannose-Sepharose gel was prepared by covalently linking D-mannose to Sepharose CL-6B using the bivalent linker divinyl sulphone [39].

2.2 Alliinase Purification

Alliinase with a specific activity of 480 U/mg was

purified from garlic cloves as described previously [6, 12].

(1) Alliinase activity

Alliinase activity was assayed by monitoring the reaction of allicin (the product of the enzymatic reaction, Fig. 1) with NTB at 412 nm [40].

(2) Preparation of the apo form of alliinase

To prepare the apo form of alliinase, a solution of the holoenzyme (5 mg/mL in PBS containing 10% glycerol) was mixed with 100 mM hydroxylamine and 20% (w/v) ammonium sulfate. The mixture was incubated at 37 °C for 3 h. After gel filtration on a Sephadex G-25 column, the apo-alliinase did not display significant absorption at 430 nm, and had negligible enzymatic activity.

(3) Reconstitution kinetics

The kinetics of reconstitution of apo-alliinase with PLP was performed by monitoring the appearance of activity using the NTB assay as follows. A 10 μM solution of apo-alliinase (~0.5 mg/mL) in 0.1 M Na phosphate, pH 7.2, was incubated with 100 μM PLP. After 1 h at room temperature the reconstituted alliinase displayed ~95% of the predicted alliinase activity.

(4) Purification of Al-ASA

The initial stages, until resuspension of the pellet after PEG-8000 precipitation, were the same as for free alliinase. Briefly, dry and peeled garlic cloves (500 g) were placed in a blender together with 750 mL of ice-cold PBS containing 20 μM PLP (buffer A) and mashed for 60 s. The slurry obtained was filtered through two layers of cheesecloth to remove the insoluble solids, and the cheesecloth was squeezed to recover as much liquid as possible. The pooled liquid was centrifuged in a Sorvall centrifuge at 20,000 × g for 20 min at 4 °C, and the insoluble pellet was discarded. The volume recovered was 900 mL. Polyethylene glycol 8,000 was added to the supernatant to 25% (w/v), and the mixture was stirred slowly for 20 min at 4 °C to allow the proteins to precipitate. It was then centrifuged at 20,000 × g for 15 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in 300 ml PBS containing 1 M ammonium sulfate, pH 6.5 (buffer B). The suspension was centrifuged at 10,000 × g for 15 min, loaded onto a mannose-Sepharose column (2.2 × 50 cm) equilibrated with buffer B, and washed with the same buffer until A_{280} was < 0.05. The column was then washed with buffer B containing 0.4 M mannose (Buffer C) to elute the fraction of the alliinase that was not complexed with ASA1. The Al-ASA was then eluted with PBS containing 0.4 M mannose (Buffer D). The preparation thus obtained was loaded onto a phenyl-Sepharose column in the presence of 1M ammonium sulfate in PBS. After washing the column with PBS containing 0.5 M ammonium sulfate, the Al-ASA was eluted from the column with 20 mM Na phosphate, pH 7.2. The eluate was placed in a Centricon device to remove low molecular weight solutes and to concentrate the complex. An alternative final purification step was SEC on a HiLoad 26/60 Superdex 200 prep grade column equilibrated with PBS. The purified Al-ASA could be stored in the presence of 0.2 M mannose at least 1 month at 4 °C, or 1 year at -20 °C, without significant loss of activity or as a lyophilized powder.

(5) Aggregation measurements

Aggregation of alliinase was monitored at 57 °C by measuring the turbidity at 400 nm, at a final protein concentration of 1 mg/mL in PBS, in the absence and presence of osmolytes.

(6) Determination of thermal stability

The time course of thermal inactivation of alliinase was measured by incubation of the enzyme (0.1 mg/mL in PBS) at 40, 50, 60 and 70 °C. Enzymic activity decreased by < 2% at 40 °C over 3 h. At 70 °C activity dropped to 0 within < 5 min (data not shown). We selected 57 °C as a suitable temperature for analysis of the stability of alliinase in the presence and absence of osmolytes. At 5, 10, 15, 30, 60, 90, 120, 140 and 180 min aliquots were cooled immediately in an ice bath, and residual activity was

measured at room temperature.

(7) Protein assay

Protein concentrations were determined by the Lowry procedure, and the concentration of pure alliinase was measured at 280 nm, using ε_{280} = 77,000 M^{-1} cm^{-1} [3].

(8) Circular dichroism

CD (circular dichroic) measurements were performed in a Jasco J-500A automated recording spectropolarimeter (Jasco Instruments, Easton, MD). The instrument was continuously purged with nitrogen before and during the experiments. Slits were programmed to yield a 10Å bandwidth at each wavelength. The CD spectra obtained were plotted as molar ellipticity values ([Θ] deg·cm^2·$dmol^{-1}$), assuming a relative Mr of 103,000 for the purified alliinase, based on a mean residue weight of 110.24. All CD spectra were recorded at RT in 50 mM sodium phosphate, pH 7.2, using the same buffer as a blank. The protein concentrations used were 0.4 and 0.5 mg/mL^{-1} for holo- and apo-alliinase respectively.

(9) Differential scanning calorimetry

DSC measurements were performed on a MicroCal MC-2D differential scanning microcalorimeter MC-2D (Malvern Instruments, Malvern, UK), with cell volumes of 1.22 mL, interfaced with an IBM-compatible computer, as described previously [34]. Data were analyzed using a kinetically controlled two-state model in which only the native state, N_2 (native dimer), and the final (irreversibly denatured) state, D_2 (denatured dimer), are significantly populated, and conversion from the native to denatured state is determined by a strongly temperature-dependent, first-order rate constant (k) that changes according to the Arrhenius equation:

$$k = \exp[E_A(1/T^* - 1/T)/R] \quad (1)$$

where E_A is the energy of activation, T^* is the temperature at which the rate constant equals 1 min^{-1}, and R is the gas constant. In a system behaving according to this equation, the rate of increase in the concentration of D_2 (and of the corresponding decrease in the concentration of N_2) is determined by the free energy of activation [33]. For this case, the excess heat capacity, C_p^{ex}, is given by the following equation [33]:

$$C_p^{ex} = \frac{1}{\nu}\Delta H \exp\left\{\frac{E_A}{R}\left(\frac{1}{T^*} - \frac{1}{T}\right)\right\} \times \exp\left\{-\frac{1}{\nu}\int_{T_o}^{T}\exp\left[\frac{E_A}{R}\left(\frac{1}{T^*} - \frac{1}{T}\right)\right]dT\right\} \quad (2)$$

where $\nu = dT/dt$ ($K \cdot min^{-1}$) is the scan-rate, and ΔH is the enthalpy difference between the denatured and native states.

If the thermal denaturation process can be described by a first-order reaction, then it can also be analyzed by use of the rate equation derived from the conventional transition state theory [41], which assumes an equilibrium constant, $K^{\ddagger}$, to exist between the reactants and the activated complex. According to this theory the rate constant is given by $k = (k_B T/h)\, K^{\ddagger}$, where k_B is the Boltzmann constant, and h is the Planck constant. The rate constant is then

$$k = \frac{k_B T}{h}\exp\left(\Delta^{\ddagger}S^{\circ}/R\right)\exp\left(-\Delta^{\ddagger}H^{\circ}/RT\right) = \left(\frac{k_B T}{h}\right)\exp(-\Delta^{\ddagger}G^{\circ}/RT) \quad (3)$$

where $\Delta^{\ddagger}S^{\circ}$, the entropy of activation, is the standard molar change of entropy when the activated complex is formed from reactants, $\Delta^{\ddagger}H^{\circ}$, the enthalpy of activation, is the corresponding standard molar change of enthalpy, and $\Delta^{\ddagger}G$ is the standard molar Gibbs free energy activation for the conversion of reactants into the activated complex.

3. Results and Discussion

3.1 Effect of Removal of PLP on the Structure of Allinase

Alliinase exists as an internal aldimine of Lys251 with the cofactor, PLP. We earlier isolated the PLP-bearing peptide, FTMSXF, corresponding to residues 247-252 of the enzyme. The X-ray structure of holo form of alliinase confirmed the assignment of

Lys251as the residue to which the cofactor is attached [7]. Numerous literature data demonstrate that binding of PLP imparts considerable role for a stability and proper conformation of most PLP-dependent enzymes [see 42 and references therein]. Among the PLP-dependent enzymes, aspartate aminotransferase (EC 2.6.1.1) and tryptophan synthase (EC 4.2.1.20) are the most widely studied enzymes with respect to their oligomeric state and the role of PLP in maintaining the structure [43-45]. In the case of tryptophan synthase removal of PLP led to the dissociation of the native enzyme into α and β subunits which could be reconstituted upon the addition of PLP [44]. However, the effect of the cofactor may considerably vary. For instance, addition of PLP to the apoenzyme sheep serine hydroxymethyltransferase did not affect the tertiary structure of the enzyme but prevented its dissociation into subunits that results in alteration of the oligomeric structure of the enzyme [46]. In the case of alliinase, comparison of the 3D structures of apo- and holo-alliinase revealed only slight rearrangements in the positions of the residues lining the PLP-binding site [7]. Fig. 2A displaying the far- and near-UV CD spectra of holo- and apo-alliinase revealed that removal of PLP did not have a major effect on the structure of the enzyme.

3.2 Calorimetric Measurements on holo- and apo-alliinase

The thermal denaturation of both holo- and apo-alliinase at pH 7.5 gave rise to well defined DSC transitions (Fig. 2B). Denaturation was irreversible for both, since no thermal transitions were observed upon rescanning of the samples and scan rate dependent (not shown) that is characteristic of an irreversible, kinetically controlled process [33, 35]. Furthermore, exothermic parts of the transitions were clearly evident for both samples, due to protein aggregation. As was done earlier [47] for transitions distorted by

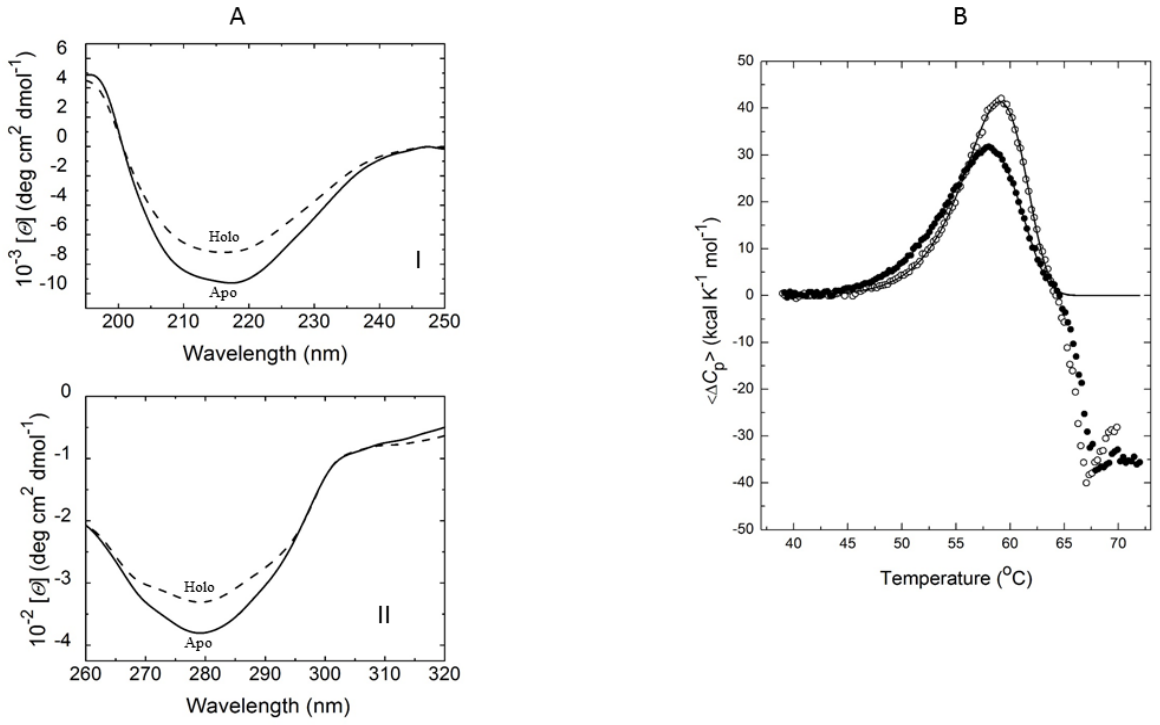

Fig. 2 (A) CD spectra of holo- and apo-alliinase in the far-(I) and near- (II) UV. (B) Temperature dependence of the excess molar heat capacity of holo- (○) and apo- (●) alliinase in 10 mM sodium phosphate, pH 7.2, at a scan rate of 1 K·min^{-1}. Solid lines are the best fit to the experimental data points using Eq. (2). The protein concentration was 1 mg·mL^{-1}.

aggregation, we used their initial parts up to a temperature 2-3 °C above the T_m (temperature of the excess heat capacity maximum), after which the full contour was obtained by extrapolation, in accordance with Eq. (2) (see Materials and Methods, 2.11.). The results of fitting the experimental data (symbols) to the simplest two-state kinetic model [33, 35] are displayed in Fig. 2B and presented in Table 1.

[a]The correlation coefficient (r) that is used as the criterion for accuracy of fitting was calculated as $r = \sqrt{1 - \sum_{i=1}^{n}\left(y_i - y_i^{calc}\right)^2 / \sum_{i=1}^{n}\left(y_i - y_i^{m}\right)^2}$, where y_i and y_i^{calc} are, respectively, the experimental and calculated values of C_p^{ex}; y_i^{m} is the mean of the experimental values of C_p^{ex} and n is the number of points. Thermograms as well as CD data also did not reveal major differences between the apo and holo forms of the enzyme (Table 1). These data support the notion that the structure of the apoenzyme remains essentially unchanged after removing the cofactor, and that binding of PLP does not induce significant conformational changes in the enzyme's secondary and tertiary structures. The X-ray data also do not reveal an observable shift in the relative positions of the large and small domains of alliinase [7]. Based on these data one can conclude that the protein structure of alliinase appears to be strictly folded and predisposed to bind PLP, and thus should not demand large conformational perturbations for holoenzyme formation.

3.3 Effects of Osmolytes on Aggregation and Thermal Inactivation of Alliinase

Already when it was first studied, alliinase was found to quickly lose activity in aqueous solution, but was more stable in the presence of glycerol [19]. Consequently, most experiments on alliinase are performed in the presence of 10% glycerol [3, 19, 21, 22]. Alliinase possesses a high propensity for aggregation. We thus considered it plausible that the stabilization of alliinase by glycerol is due to its known osmolytic properties. Carbohydrates account for ~77% of the dry weight of garlic bulbs [8]. We considered, therefore, that it would be worth checking the effect of polyol osmolytes on alliinase stability. In the present study we investigated in detail the influence of glycerol and several other commonly used polyol osmolytes on the stability of the enzyme and on its tendency to aggregate. Osmolytes affect the balance between the intramolecular interactions of the protein's functional groups and their interactions with the solvent environment [26, 48-50]. They can substitute for water on the surface of a protein, producing a crowding effect on the structure, thus favoring a more compact polypeptide conformation that stabilizes it in its folded state [26, 30].

Fig. 3A shows that the aggregation of alliinase is significantly suppressed in the presence of glycerol, sucrose and sorbitol. To assess their influence on alliinase's stability, we studied their effect on its thermal inactivation. Fig. 3B shows that all three osmolytes stabilize the enzyme, and retard its thermal inactivation. In this case, glycerol and sorbitol both had a stronger protective effect than sucrose (Fig. 3B). The effects of osmolytes on thermal denaturation were also studied using DSC. The DSC data, presented in Tables 2 and 3 and in Fig. 3 indeed confirm their stabilizing effect. As mentioned above, thermal denaturation of alliinase is irreversible. One possible explanation for the fast and irreversible denaturation could be relatively easy entry of denaturing agents into the interior of the protein through numerous water channels. The extent of alliinase to solvation and accessibility to water may correlate with its stability strongly depends on the presence of cosolvents that may substitute water bound to protein [27, 29]. Preferential dehydration of enzyme as well as crowding effects likely provide by glycerol and other osmolytes to contribute to its stability [27, 29]. Thus, we showed that polyol osmolytes like sucrose, sorbitol, and glycerol reliably prevent the aggregation and effectively stabilize allinase without substantial changes

Table 1 Arrhenius equation parameter estimates for apo- and holo-alliinase.

Parameter	Holo-alliinase	Apo-alliinase
E_A (kcal·mol^{-1})	76.6 ± 1.3	63.6 ± 1.9
T^* (^{o}C)	62.7 ± 0.2	62.4 ± 0.3
r	0.9971	0.9945

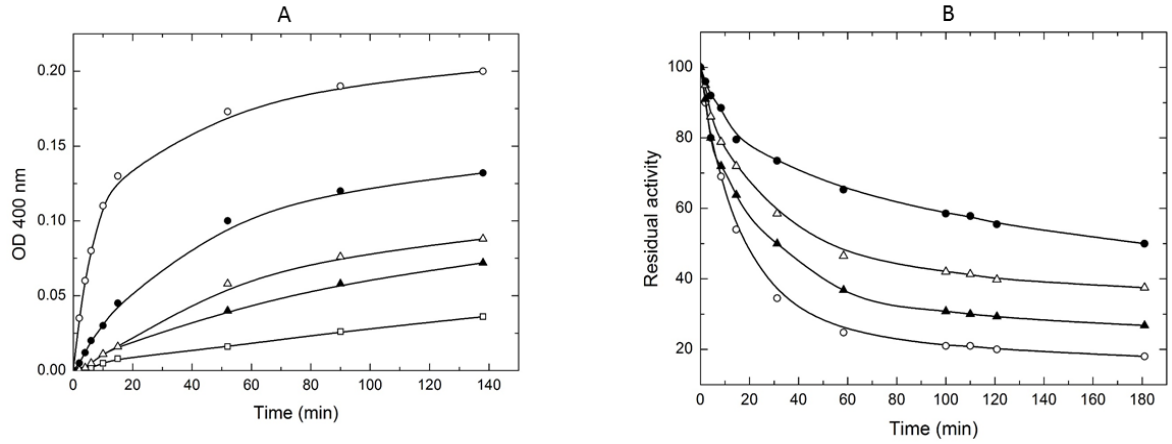

Fig. 3 (A) Effect of polyol osmolytes on aggregation of alliinase alone (○), and in the presence of 1 M glycerol (●), 2 M glycerol (Δ), 1 M sorbitol (▲) and 1 M sucrose (□), at 57 °C. (B) Effect of polyol osmolytes on thermal inactivation of alliinase alone (○), and in presence of 1 M glycerol (●), 1 M sorbitol (Δ) and 1 M sucrose (▲), at 57 °C.

its function.

3.4 DSC Studies of the Al-ASA

As already mentioned, effective application of alliinase for therapeutic treatments will require its stabilization. Since it is not feasible to administer high concentrations of an osmolyte together with the enzyme, we considered as a plausible alternative administration of alliinase as its tight complex with ASAI. Accordingly, we used DSC to assess the degree of stabilization conferred on alliinase by its complexation with ASAI. As in the calorimetric measurements of holo- and apo-alliinase presented above, in the presence and absence of osmolytes, thermal denaturation of Al-ASA gave rise to well-defined DSC transitions distorted by aggregation. Fig. 4 shows that the thermal stability of alliinase is higher when it is in the Al-ASA form.

The excess heat capacity functions were analyzed by fitting the experimental data to Eq. (2) for the two-state irreversible model (see Materials and Methods, 2.11.). The results of this fitting are shown in Fig. 3 (solid lines) and in Table 2.

The value of *r* was better than 0.9972 in all fittings.

The Arrhenius parameters permit estimation of the enzyme's stability in terms of values of the denaturation constants at different temperatures (see Fig. 4), and calculation of the $t_{1/2}$ values for denaturation (Table 3).

Moreover, since denaturation of alliinase is a first-order reaction, we analyzed this process further in a thermodynamic form of the rate equation obtained from conventional transition state theory (see Eq. (3) in Materials and Methods, 2.11.). Results of this analysis are shown in Table 2. Thus, use of DSC permitted demonstration of the stabilization of alliinase by ASAI. It can be seen from Fig. 3 that ASAI is more effective than the most commonly used osmolytes, TMAO and sorbitol (Fig. 3). It is interesting that although the lectin complex is more stable than alliinase in 10% glycerol below 28 ^{o}C, above this temperature the opposite is true (Fig. 4).

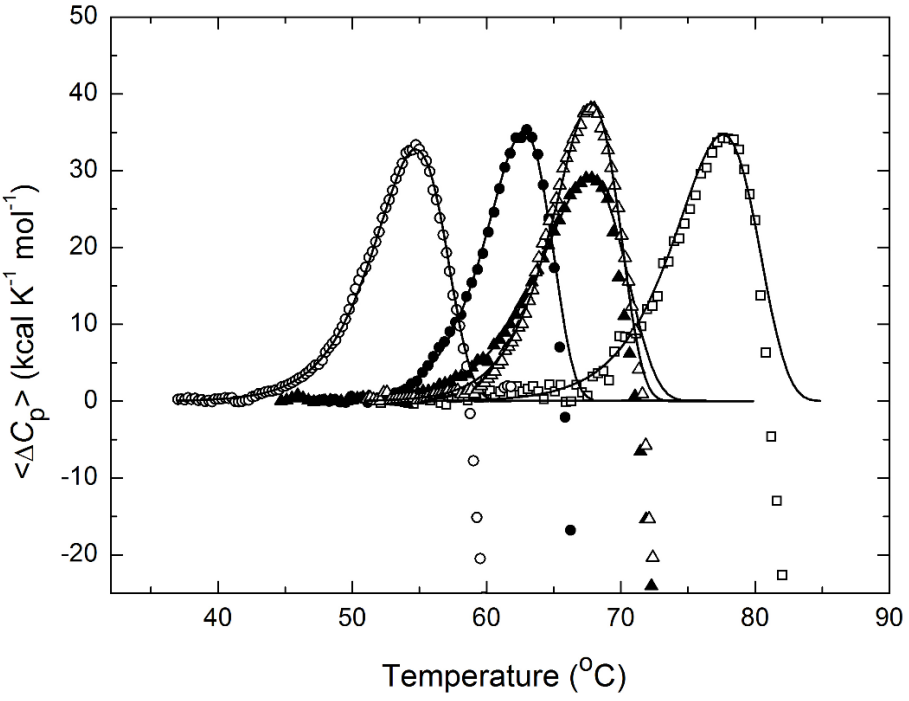

Fig. 4 Temperature dependence of the excess molar heat capacity of alliinase (10 μM) alone (○), and in the presence of 1 M TMAO (●), 1 M sorbitol (▲), 1 M glycerol (□), and 10 μM Al-ASA (Δ). The scan rate was 1 K·min^{-1}. Solid lines are the best fit to the experimental data points using Eq. (2). The alliinase concentration was 1 mg·mL^{-1}. The buffer employed was 10 mM sodium phosphate, pH 7.2.

Table 2 Arrhenius and Eyring parameter estimates for the two-state irreversible model of alliinase denaturation.

Function	Alliinase	Alliinase + 1 M TMAO	Alliinase + 1 M Sorbitol	Al-ASA	Alliinase + 1 M Glycerol
E_A (kcal·mol^{-1})	80.3 ± 1.3	92.8 ± 1.6	73.1 ± 1.8	94.5 ± 1.9	78.3±1.9
T^* (^{o}C)	57.4 ± 0.2	65.0 ± 0.2	69.2 ± 0.3	70.0 ± 0.2	79.8 ± 0.3
$\Delta^{\#}H_{25^oC}$(kcal·mol^{-1})	24.7 ± 1.3	27.8 ± 1.6	26.3 ± 1.8	29.3 ± 1.9	29.0 ± 1.9
$\Delta^{\#}S_{25^oC}$(cal·K^{-1}·mol^{-1})	82.9 ± 0.5	93.3 ± 0.5	88.3 ± 0.4	98.4 ± 0.4	97.3 ± 0.4
$\Delta^{\#}G_{25^oC}$(kcal·mol^{-1})	25.3 ± 1.3	28.4 ± 1.6	26.9 ± 1.7	29.9 ± 1.4	29.6 ± 1.3

Table 3 Half-life times for thermal denaturation of alliinase.

Temperature (^{o}C)	Alliinase	Alliinase + TMAO	Alliinase + Sorbitol	Al-ASA	Alliinase + Glycerol
	$t_{1/2}$ (min)				
10	5.8E7	3.3E11	4.1E9	4.3E12	6.3E11
20	4.3E6	1.2E9	5E7	1.4E10	5.8E9
30	46210	6.3E6	787667	6.3E7	6.9E7
40	693	46210	16120	433217	1.1E6
50	12	433	433	3872	21661
60	0.3	5.8	13.9	46.2	533.2
70	0.008	0.1	0.6	0.7	17.3
80	3E-4	0.002	0.03	0.01	0.67
90	1.2E-5	5.3E-5	0.002	7E-4	0.031

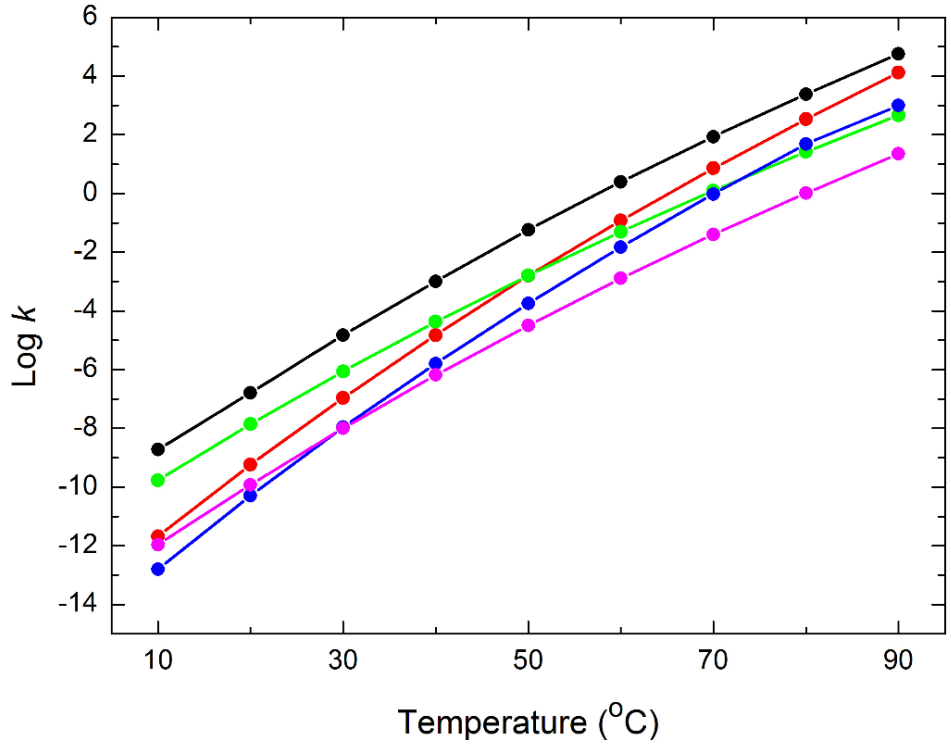

Fig. 5 Temperature dependence of the first-order rate constant for thermal denaturation of alliinase alone (●), and alliinase in presence of TMAO (●), sorbitol (●), glycerol (●) as well as in complex with ASAI (●).

The results obtained show a significant increase in the stability of the complex relative to the free enzyme (Tables 2 and 3), demonstrating that Al-ASA may considered as a naturally existing stable active form of alliinase. Use of alliinase for allicin synthesis in biotechnology necessitates having the protein in a stabilized active form. Osmolytes certainly can contribute to this goal, but Al-ASA seems to provide a more attractive alternative. This form is convenient to store, and can be easily lyophilized practically without loss of activity.

4. Conclusions

In the present study it was shown that binding of the cofactor PLP to the holoenzyme form of alliinase does not contribute significantly to its stability. Osmolytes, which act as chemical chaperones, stabilize alliinase against thermal denaturation, retarding its unfolding and subsequent aggregation. The mannose-specific lectin, ASAI, which occurs alongside alliinase in garlic, stabilizes the enzyme, which is a glycoprotein, by interacting tightly with mannose residues on the protein's surface. Since the ASAI/alliinase complex remains catalytically active, it can be effectively use in biotechnological and medical applications of this otherwise unstable enzyme. The further characterization of this complex will help to develop new formulations of alliinase, which will promote to apply various therapeutic activities of allicin.

Declarations

The authors declare no conflict of interest.

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