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Crystallization, solubility and thermodynamics of the highly thermostable glucose isomerase from *Streptomyces* sp. strain

Mohamed A. Borgi[1,2], Moez Rhimi[3] and Adel Kadri[4]

[1] *Laboratory of Enzymes and Metabolites of Prokaryotes, CBS, B.P 1177, Sfax, Tunisia*
[2] *Department of Life Sciences, Unit of Macromolecular Biochemistry and Genetic, Faculty of Sciences of Gafsa, Zarroug 2112, Gafsa, Tunisia*
[3] *INRA, UMR 1319 Micalis, F-78350 Jouy-en-Josas, France*
[4] *Laboratory of Plant Biotechnology Applied to Crop Improvement, Faculty of Sciences of Sfax, B.P. 1171, 3000 Sfax, University of Sfax, Tunisia*

Abstract. The crystallization behaviour of the highly thermostable glucose isomerase from the *Streptomyces* sp. strain isolated from Tunisian soil was investigated using ammonium sulfate as a precipitating agent. We established phase diagrams at different temperatures and protein concentrations. It was found that the solubility increased with increasing temperature and decreased with increasing salt concentration. The temperature-dependent solubility was used to characterize the thermodynamic parameters of crystallization such as enthalpy, entropy and free energy.

Key words: Glucose isomerase — Biocrystallization — Solubility — Phase diagrams — Thermodynamics

Introduction

Macromolecular crystallization, which includes the crystallization of proteins, nucleic acids, and larger macromolecular assemblies such as viruses and ribosomes, is based on a rather diverse set of principles, experiments and ideas (McPherson 2004; Srimahaprom and Flood 2013). It is very often the most difficult and time-consuming step in the determination of a protein's atomic structure. The basics of protein crystallization conform to the classical understanding of small molecules crystallisation, with the main difference from small molecule crystal growth being the consequent greater sensitivity of proteins to external conditions (Ducruix and Giegé 1999). Protein structure determination has long been considered to be an important tool, by allowing for example the design of novel drugs on the basis of the structures of protein therapeutic targets (Lee et al. 2005), and therefore plays a key role in structure based drug discovery and structural genomics projects (Chayen 2004). Crystallization of macromolecules remains a problematic and complex process owing to the large size and the frequent flexibility of these molecules often composed of several subunits. Difficulties are also attributed to their relatively chemical and physical instable state due to the unfolding, hydration requirements, temperature sensitivity and dynamic properties (Bergfors 1999; McPherson 1999).

Knowledge of a protein's solubility which depends on solution variables, such as temperature, and ionic strength, is important for defining optimum conditions for protein crystal growth. Indeed, it is an important thermodynamic parameter which reflects intermolecular interactions occurring between the macromolecules and the solvent components. In order to find optimal crystallization conditions for glucose isomerase (GI) its solubility behaviour as a function of different parameters including protein and salt concentration as well as temperature has been characterized. Numerous experiments point out that most proteins are difficult to crystallize (Durbin and Feher 1996) and even if a protein tends to crystallize relatively easily, there are many parameters that must be taken into account. Successful crystallization of any given protein is determined by a combination of factors, like the presence

Correspondence to: Mohamed Ali Borgi, Department of Life Sciences, Unit of Macromolecular Biochemistry and Genetic, Faculty of Sciences of Gafsa, Zarroug 2112, Gafsa, Tunisia
E-mail: borgima@yahoo.com

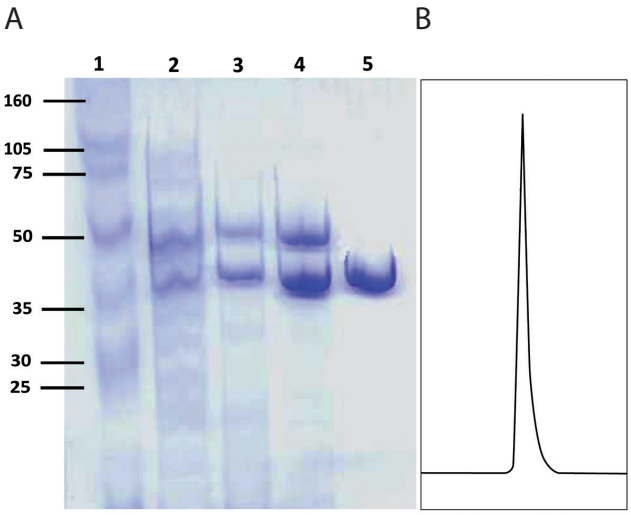

Figure 1. Coomassie brilliant blue-stained SDS-PAGE gel (**A**) and size exclusion HPLC chromatogram (**B**) of the purified glucose isomerase. Lane 1, protein markers (molecular masses (in kD) are shown on the left). Lane 2, crude cell extract. Lane 3, crude extract after heat treatment. Lane 4, sample from lane 3 after ammonium sulfate precipitation. Lane 5, purified glucose isomerase. Gel filtration chromatogram was obtained by using the Bio-Sil SEC 250-5 (30 X 0.78 cm) column from BIO-RAD.

of an adequate degree of protein supersaturation, along with a particular set of empirically selected solution conditions that enable a productive kinetic pathway for nucleation and crystal growth (Bolen 2004). The prerequisite for protein crystal growth is the transfer of a purified protein in solution to the status of supersaturation. In addition, conditions such as pH, temperature and ionic strength must be chosen correctly to avoid enzyme precipitation. Better knowledge of the influence of those parameters on the solubility behaviour is thus essential in view of crystal structure determination (Chayen 1999), as well as for protein engineering to optimize biocatalysts for industrial applications (Rosenbaum 1999; Stewart 2002). Finding conditions in which biological macromolecules form suitable crystals are recognized as one of the major bottlenecks in structural biology.

This work focuses on the crystallization of the GI characterized from the thermophilic *Streptomyces* sp. strain, isolated from a Tunisian hot spring soil. This enzyme is interesting since its amino-acid sequence is attractive due to the abundance of hydrophobic residues and some original amino-acid substitutions, which distinguish it from other reported GIs (Borgi et al. 2004, 2009). Moreover, we also explored the effect of salt concentration and the enzyme concentration in the range of 8–14 mg/ml on the solubility of GI at different temperatures. The obtained solubility data contribute to clarify the thermodynamic behaviours such as enthalpy, entropy and free energy of this GI as a relevant industrial biocatalyst.

Materials and Methods

Purification of glucose isomerase

The thermostable GI from the isolated thermophilic *Streptomyces* sp. strain (from a Tunisian hot spring soil) was purified based on the protocol previously described (Borgi et al. 2004). Hence, the enzyme was purified to homogeneity by using a heat treatment step, followed by ammonium sulfate ($(NH4)_2SO_4$) precipitation and fast-performance liquid chromatography (FPLC). The purity level of the GI was examined by SDS-PAGE and size exclusion HPLC chromatography, as can be seen in Figure 1. The enzyme concentration was determined using Bradford's method with bovine serum albumin as the standard (Bradford 1976).

Sample preparation

In all experiments, the solutions were prepared with ultrapure sterile water and filtered through membranes with a 0.22 μm pore size. A buffer containing 100 mM Tris-HCl (Sigma-Aldrich) was adjusted at pH 8.0 with concentrated solutions of hydrochloride acid. A stock solution of ammonium sulfate, 20 mM $MgCl_2$ and 2 mM $CoCl_2$ (Sigma-Aldrich) was prepared with ultrafiltered, deionized water. Sodium azide at a concentration of 0.1% (w/v) was added to all buffer solutions as an antimicrobial agent. The concentration of the protein stock solution was 14 mg/ml.

Glucose isomerase crystallization

The crystallization behaviour of the protein was screened by the hanging drop method, which was initiated by mixing one volume of protein with one volume of reservoir solution and by changing the following parameters: concentrations of both protein and precipitant, and temperature at constant pH. The protein solution used in the crystallization set-up contained the enzyme in 100 mM Tris-HCl at pH 8.0, 20 mM $MgCl_2$ and 2 mM $CoCl_2$. The protein concentration ranges varied from 8 to 14 mg/ml by increment of 2, and the ammonium sulfate concentration ranged from 0.5 to 1.75 M by increment of 0.25. The multi-wells were placed in incubators at different temperatures (10 to 35°C by increment of 5). Crystallization assays were performed by using *EasyXtal* Tool crystallization plates (QIAGEN). The drop contained 2 μl of enzyme solution mixed with 2 μl of reservoir solution and equilibrated over reservoir. Plates were stored at different temperatures for durations depending on the crystallization conditions. An example of the obtained crystals was shown in Figure 2.

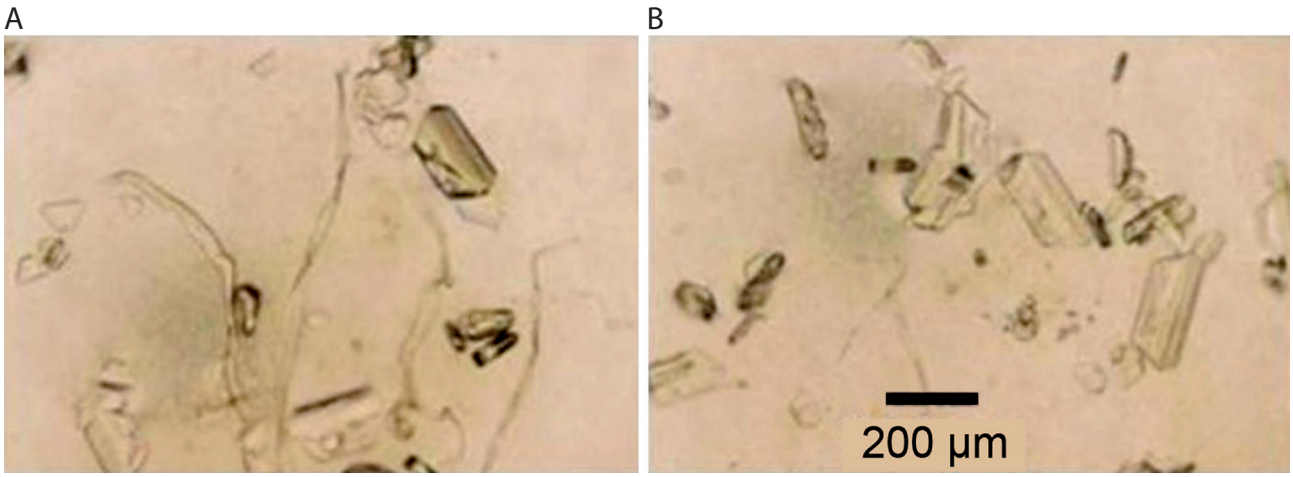

Figure 2. Crystals of glucose isomerase obtained at 14 mg/ml of protein with 1.50 M (**A**) and 1.75 M (**B**) of ammonium sulfate. Crystals belong to the space group $I4_1$ with unit cell parameters of: a = 128.221 Å, b = 128.221 Å, c = 171.326 Å and β = 90°.

Solubility measurement

The protein concentration, in equilibrium with the crystals, which is called solubility (S) was measured according to the following scheme. Crystals were grown using the hanging drop, which contained a total volume of 4 µl (2 µl of enzyme solution mixed with 2 µl of reservoir). Withdraw 1 or 2 µl of clear supernatant under the binocular. If too microcrystals are present, the drop was centrifuged and the aliquot was taken from the clear supernatant to prevent crystals. Therefore, dilution was made to the minimal volume required for optical density (OD_{280}) measurements allowing solubility determination. The solubility measurements were followed by spectrophotometry until it remained unchanged for at least three weeks. The supernatant was then filtered through a membrane with a pore diameter of 0.22 µm and the absorbance of the filtrate was calculated using the extinction coefficient of the enzyme, which is 1.12 $ml \cdot mg^{-1} \cdot cm^{-1}$ at 280 nm. The experimental error was estimated about 15%.

Results and Discussion

Effect of precipitating agent concentration on the solubility

Solubility (S) is defined as the concentration of soluble protein in equilibrium with the crystalline state at a given temperature, pH value and in the presence of a given concentration of solvent. Probing the solubility of the proteins at different concentrations with different precipitating agent concentration is useful to determine the solubility (or phase) diagram. Phase diagrams plotting the solubility *versus* temperature (from 283 to 308 K) at various precipitating agent (from 0.5 to 1.75 M) and protein (8–14 mg/ml) concentrations have been established experimentally. As shown in Figure 3, the GI exhibited retrograde solubility dependence as a function of precipitating agent concentration, indicating that attraction is induced between protein molecules in the presence of ammonium sulfate. The rate of decreasing solubility as a function of ammonium sulfate concentration was more pronounced at lower temperatures (i.e., for GI concentration of 14 mg/ml, the solubility variation could be reduced by a factor of ~14 and ~5 at 283 and 293 K, respectively). The solubility decreases with increasing ammonium sulfate concentration by a factor of ~2.3, ~1.4 and ~1.2 at 298, 303 and 308 K, respectively, whatever the GI concentration. Our results are in agreement with those reported concerning the crystallization of GI from other strains. In fact, Chayen et al. (1988), by using the hanging drop method with ammonium sulfate as a precipitant agent, found that the solubility of crystalline GI from *Arthrobacter* decreased with increasing ammonium sulfate concentration. Furthermore, Vuolanto et al. (2003) studied the solubility of GI from *Streptomyces rubiginosus*. They found that the solubility decreased logarithmically when increasing the ammonium sulfate concentration. However, for low magnesium sulfate concentrations, GI crystals have a lower solubility. Using laser confocal differential interference contrast microscopy and with Polyethylene glycol (PEG) as a precipitant agent for the crystallization of GI from *Streptomyces rubiginosus*, Sleutel et al. (2009) found a decrease in the solubility with increasing PEG concentration. Van Driessche et al. (2009) described the solubility of the same GI using ammonium sulfate at 0.6 and 0.85 M with the higher solubility obtained at 0.6 M of ammonium sulfate. Indeed, at low salt concentrations the dominating effect is screening of negative patches

on the protein surface by sulfate ions, which would reduce the charge repulsion between the protein molecules, and therefore lead to the observed drop in solubility (Einsenhaber 1999).

Effect of temperature on the solubility

Biological macromolecules can be crystallized by using a variety of techniques, and a wide range of reagents which produce supersaturated mother liquors. These may, in turn, be applied under different physical conditions such as temperature, which can be an important variable in biological macromolecule crystallization, since an understanding of the phase diagram is useful for the production of suitable crystals for structural biology (Boistelle et al. 1992; Lafont et al. 1994, 1997; Revalor et al. 2010).

The results summarized in Figure 3 show an increase in the solubility of GI with increasing temperature. In the GI concentration range of 8–14 mg/ml, an increase in temperature from 273 to 308 K was found to affect more the solubility of

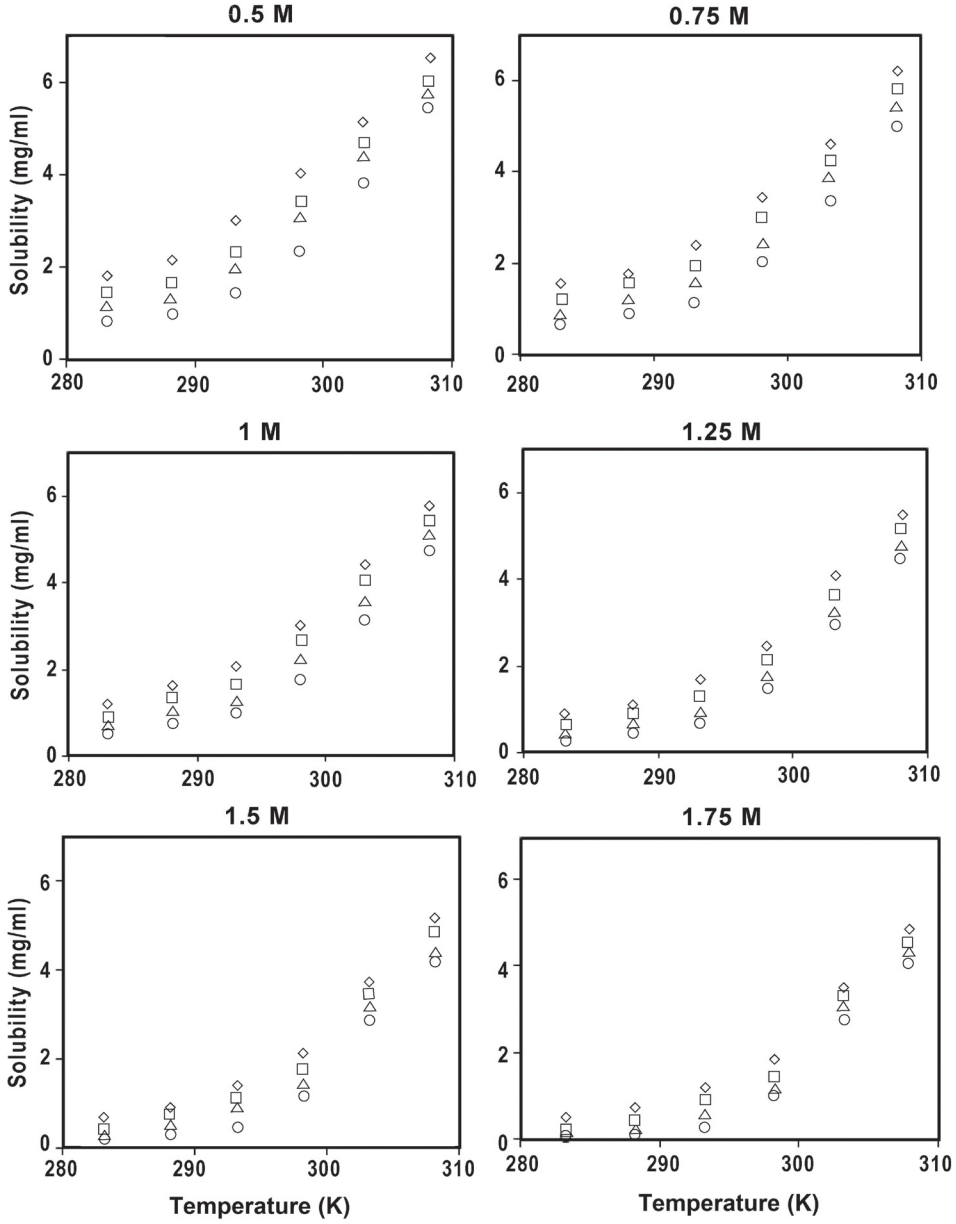

Figure 3. Solubility curve of GI as a function of temperature in 0.5 M, 0.75 M, 1 M, 1.25 M, 1.50 M and 1.75 M of ammonium sulfate. Solubility's data were obtained at 14 mg/ml (diamonds), 12 mg/ml (squares), 10 mg/ml (triangles) and at 8 mg/ml of GI (circles).

GI crystals at higher salt concentration (i.e., S increased by a factor of ~5 at 0.5 M and ~33 at 1.75 M for a GI concentration of 10 mg/ml). These results reinforced what was so far reported for the crystallization of several GI. In fact, Visuri et al. (1990) determined the solubility of GI *Streptomyces rubiginosus* at temperature range from 290 to 305 K and found that the solubility increased as increasing temperature at 0.84 M of ammonium sulfate. Similar data were described by Van Driessche et al. (2009) where they observed an enhancement in the solubility of GI with temperature at concentrations of 0.6 M and 0.85 M ammonium sulfate.

Enthalpy, entropy and free energy of crystallization

From solubility data, thermodynamic parameters are often calculated using the expression for the standard free energy ΔG^0 of crystallization and the equilibrium constant of crystallization K_{cryst} (van Holde et al. 2006; Taulelle et al. 2009)

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT \ln K_{cryst} \quad (1)$$

$$K_{cryst} = a_e^{-1} = \left(\gamma_e \frac{S}{C^0}\right)^{-1} \quad (2)$$

where ΔH^0 and ΔS^0 are the standard enthalpy and entropy of crystallization, respectively. T is the temperature, R = 8.314 J/mol/K is the universal gas constant, a_e is the activity of GI in solution in equilibrium with the crystal, γ_e is the corresponding activity coefficient, S is the equilibrium protein concentration, and C^0 is the concentration of the solution in chosen standard state (1 mol/kg). If we assume ideal solution (i.e. activity coefficient 1) (Sleutel et al. 2009), the Eq. 2 can be written (Atkins and De Paula 2006)

$$K_{cryst} \approx \left(\frac{S}{C^0}\right)^{-1} \quad (3)$$

From Eq. 1 it follows that

$$\ln\left(\frac{S}{C^0}\right) = \frac{\Delta H^0}{RT} - \frac{\Delta S^0}{R} \quad (4)$$

To estimate ΔH^0, ln (S/C^0) is plotted against $1/T$ as shown in Figure 4 and fitting the data points in this plot. We get a straight line which slope is $\Delta H^0/R$ and intercept is $-\Delta S^0/R$.

The crystallization enthalpies were determined from the slopes of Figure 4. As expected, the solubility dependence on temperature yields a negative enthalpy of crystallization. The change in enthalpy of GI crystallization is negative (exothermic process) indicating a heat release when crystals were formed. As shown in Table 1, at lower ammonium sulfate concentration, the variation of GI concentration does not affect the enthalpies value. (i.e., ΔH^0 = –57 ± 9 kJ/mol at 8 mg/ml and –44 ± 6 kJ/mol at 12 mg/ml), however, a little variation was observed at higher ammonium sulfate concentration and was attributed to the predominant salting-out effect (ΔH^0 ranged from –132 ± 20 kJ/mol to –87 ± 13 kJ/mol for the same conditions). In this study, the enthalpy of crystallization tends to decrease as increasing salt concentration (i.e., at 8 mg/ml, ΔH^0 = –57 ± 9 kJ/mol and –132 ± 20 kJ/mol for ammonium sulfate concentration of 0.5 M and 1.75 M, respectively). In case of the *Streptomyces* sp GI crystals (data not shown), the obtained larger enthalpy value corresponds to the large surface energy of these crystals, as previously described for the Taka-amylase A by Ninomiya et al. (2001). Furthermore, Van Driessche et al. (2009) reported that crystallization enthalpies values for the *Streptomyces rubiginosus* GI were –144 kJ/mol and –174 kJ/mol, respectively, at 0.6 M and 0.85 M of ammonium sulfate. However, Suzuki et al. (Suzuki et al. 2002) found an enthalpy of crystallization of –160 kJ/mol at 0.91 M ammonium sulfate.

The same trend was observed for entropy with a decrease in the entropies values as increasing salt concentra-

Table 1. Thermodynamic parameters of *Streptomyces* sp. GI crystallization

GI (mg/ml)	$(NH_4)_2SO_4$ (M)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol K)
14	0.50	–38 ± 6	–
	0.75	–42 ± 6	–18 ± 3
	1.00	–47 ± 7	–32 ± 5
	1.25	–55 ± 8	–60 ± 9
	1.50	–61 ± 9	–77 ± 10
	1.75	–67 ± 10	–98 ± 15
12	0.50	–44 ± 6	–24 ± 3
	0.75	–47 ± 7	–33 ± 5
	1.00	–53 ± 8	–52 ± 8
	1.25	–62 ± 9	–81 ± 12
	1.50	–71 ± 10	–111 ± 17
	1.75	–87 ± 13	–162 ± 24
10	0.50	–44 ± 6	–43 ± 6
	0.75	–53 ± 8	–53 ± 8
	1.00	–59 ± 9	–71 ± 10
	1.25	–71 ± 10	–110 ± 17
	1.50	–84 ± 12	–151 ± 22
	1.75	–107 ± 17	–227 ± 34
8	0.50	–57 ± 9	–66 ± 10
	0.75	–61 ± 9	–76 ± 12
	1.00	–68 ± 10	–98 ± 15
	1.25	–84 ± 12	–150 ± 22
	1.50	–100 ± 15	–204 ± 30
	1.75	–132 ± 20	–307 ± 45

GI, glucose isomerase; ΔH^0, standard enthalpy; ΔS^0, standard entropy.

tion (i.e., at 8 mg/ml, ΔS^0 ranged from ~ –66 ± 10 J/mol·K to ~ –307 ± 45 J/mol·K for salt concentration range of 0.5 and 1.75 M, respectively). This decrease may be attributed to the activity of the bulk water. For the crystallization of GI from *Streptomyces rubiginosus*, Van Driessche et al. (2009) found that ΔS^0 took a value of –370 J/mol K and –462 J/mol K, respectively at 0.6 M and 0.85 M of ammonium sulfate. The results reported by Suzuki et al. (2002), indicated crystallization entropy of –420 J/mol·K at 0.91 M of ammonium sulfate. We attributed the dissimilarity in the thermodynamic values to the difference in the biochemical parameters, amino acid sequence and to the crystallization and the solubility method. We note that at lower salt concentration, the effect of protein concentration on the enthalpy and entropy was not significant.

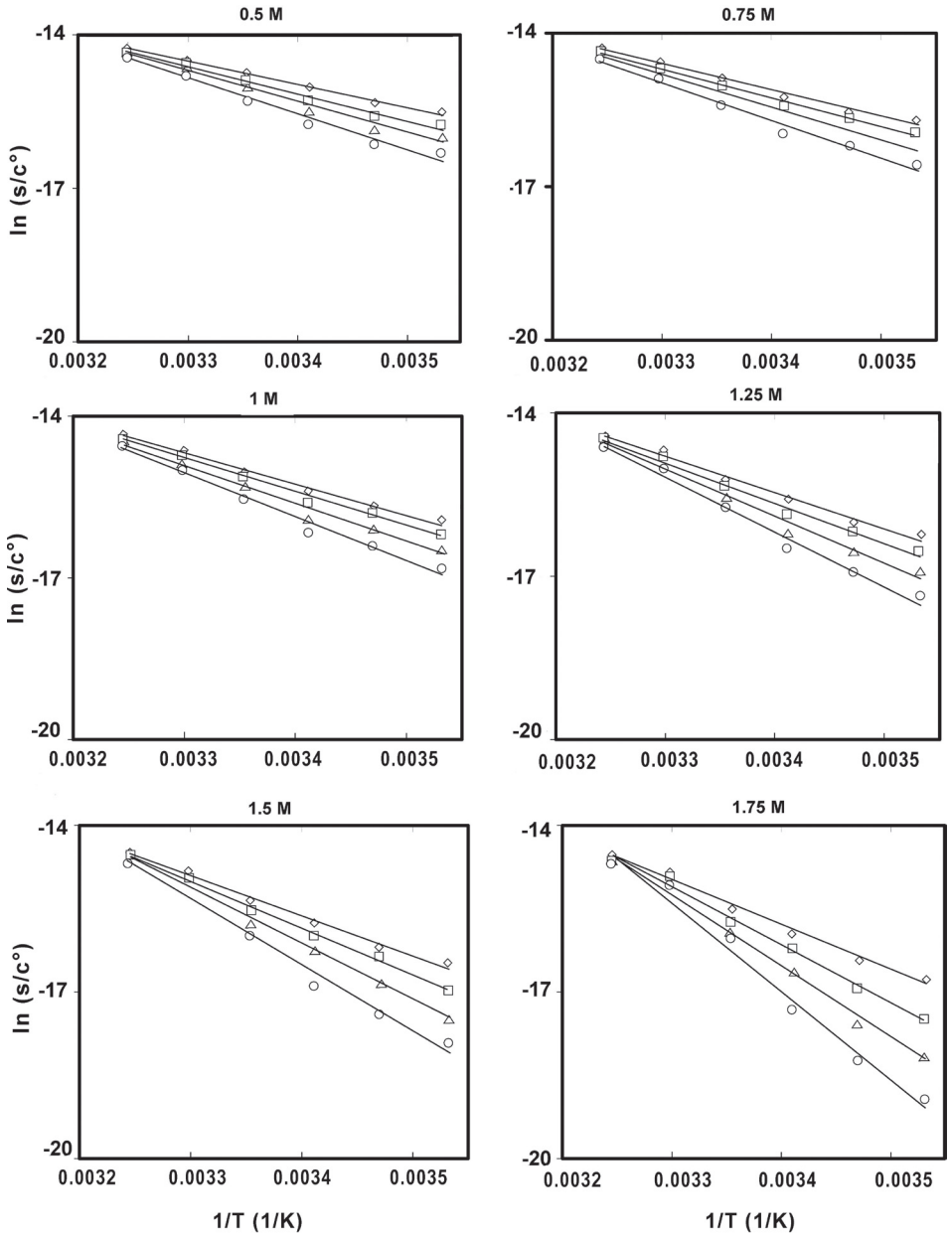

Figure 4. Van't Hoff plot of the solubility data of GI (logarithmic solubility versus reciprocal of absolute temperature) in 0.5 M, 0.75 M, 1 M, 1.25 M, 1.50 M and 1.75 M of ammonium sulfate. Solubility's data were obtained at 0.5 M of ammonium sulfate and 14 mg/ml of GI (diamonds), 0.5 M of ammonium sulfate and 8 mg/ml of GI (squares), 1.75 M of ammonium sulfate and 14 mg/ml of GI (circles) and at 1.75 M of ammonium sulfate and 8 mg/ml of GI (triangles). T, temperature; C^0, concentration of the solution in chosen standard state (1 mol/kg); S, equilibrium protein concentration.

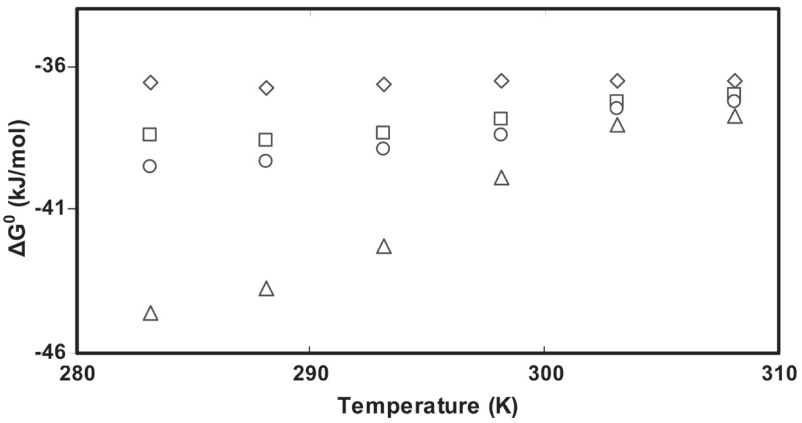

Figure 5. Temperature dependence of the crystallization free energy of GI crystals at 14 mg/ml and at 8 mg/ml. Data obtained at 0.5 M of ammonium sulfate and 14 mg/ml of GI (diamonds), 0.5 M of ammonium sulfate and 8 mg/ml of GI (squares), 1.75 M of ammonium sulfate and 14 mg/ml of GI (circles) and at 1.75 M of ammonium sulfate and 8 mg/ml of GI (triangles). ΔG^0, standard free energy of crystallization.

As previously reported by Sleutel et al. (2009), the negative entropy contribution for GI indicated an entropy loss upon the attachment of protein molecules to the crystal either due to the constrained translational and rotational degrees of freedom of these biomolecules. Similar data were reported in the case of the porcine insulin (Bergeron et al. 2003). Furthermore, it was stated that enthalpy and entropy contributions are responsible of the thermodynamics of molecular recognition during crystallization (Vekilov 2003). The effect of water/solvent molecules would have a significant role and may be explained by an entropic effect (Vekilov et al. 2002).

Combining Eqs (1) and (2) gives

$$\Delta G^0 = RT \ln \left(\frac{S}{C^0}\right) \quad (5)$$

As shown in Figure 5, the free energy of crystallization is dependent on temperature for a higher salt concentration and lower GI concentrations (i.e., at 1.75 M of ammonium sulfate and for a GI concentration of 8 mg/ml, ΔG^0 varied from –44.6 ± 6 to –37.7 ± 6 kJ/mol, however, at 0.5 M of ammonium sulfate and for GI concentration of 14 mg/ml, ΔG^0 was constant and took a value of –36.51 ± 6 kJ/mol). The gradual decrease in the absolute ΔG^0 as a function of temperature is the result of the increasing contribution of $T \cdot \Delta S^0$ which disfavoured the crystallization of enzyme. The obtained results correlate well with those previously reported by Sleutel et al. (2009) for the crystallization of GI from *Streptomyces rubiginosus*, using a PEG solution as precipitant agent.

In this study, GI crystals exhibited a retrograde solubility dependence on ionic strength and a solubility dependence on temperature. The GI solubility is affected more by varying the temperature than the ionic strength and the protein concentration. The GI crystallizes following an exothermic process with a negative crystallization enthalpy. The ΔH^0 values of crystallization, decreases with increasing salt concentration and a significant difference at lower GI concentration was observed. The lowest value of crystallization enthalpy (–132 kJ/mol) was found at higher salt concentration (1.75 M) and at lower GI concentration (8 mg/ml) which is in agreement with the salting out concept leading to large crystals with a few numbers. The entropies behaviour of GI in this study indicated a release, trapping and rearrangement of water upon attachment to the crystal, which can be defined as a hydrophobic interaction. The decrease in the absolute free energy value of crystallization is observed at higher salt concentration and for lower GI concentration with lower temperature indicating that crystallization will be promoted.

Finally, this contribution is certainly of importance since it brings additional data to how to get crystals allowing the determination of the 3D structure of this industrial GI. From such information, we will be better able to understand the origin of their interesting features, as well as the key roles of many amino-acid substitutions.

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References

Atkins P. W., De Paula J. (2006): Physical Chemistry. (8th Ed), Oxford University Press, Oxford

Bergeron L., Filobelo L. F., Galkin O., Vekilov P. G. (2003): Thermodynamics of the hydrophobicity in crystallization of insulin. Biophys. J. **85,** 3935–3942 http://dx.doi.org/10.1016/S0006-3495(03)74807-3

Bergfors T. M. (1999): Protein Crystallization, International University Line, La Jolla, CA

Boistelle R., Astier J. P., Marchis-Mouren G., Desseaux V., Haser R. (1992): Solubility, phase-transition, kinetic ripening and growth-rates of porcine pancreatic alpha-amylase isoenzymes. J. Cryst. Growth **123,** 109–120 http://dx.doi.org/10.1016/0022-0248(92)90015-B

Bolen D. W. (2004): Effects of naturally occurring osmolytes on protein stability and solubility: issues important in protein crystallization. Methods **34,** 312–322

http://dx.doi.org/10.1016/j.ymeth.2004.03.022
Borgi M. A., Belguith K. S., Ben Ali M., Mezghani M., Tranier S., Haser R., Bejar S. (2004): Glucose isomerase of the Streptomyces sp. SK strain: purification, sequence analysis and implication of alanine 103 residue in the enzyme thermostability and acidotolerance. Biochimie **86,** 561–568
http://dx.doi.org/10.1016/j.biochi.2004.07.003
Borgi M. A., Rhimi M., Aghajari N., Ben Ali M., Juy M., Haser R., Bejar S. (2009): Involvement of cysteine 306 and alanine 63 in the thermostability and oligomeric organization of glucose isomerase from Streptomyces sp. SK. Biologia **64,** 845–851
http://dx.doi.org/10.2478/s11756-009-0155-y
Bradford M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248–254
http://dx.doi.org/10.1016/0003-2697(76)90527-3
Chayen N. E., Akins J., Campbell-Smith S., Blow D. M. (1988): Solubility of glucose isomerase in ammonium sulphate solutions. J. Cryst. Growth **90,** 112–116
http://dx.doi.org/10.1016/0022-0248(88)90305-3
Chayen N. E. (1999): Recent advances in methodology for the crystallization of biological macromolecules. J. Cryst. Growth **198/199,** 649–655
http://dx.doi.org/10.1016/S0022-0248(98)01151-8
Chayen N. E. (2004): Turning protein crystallisation from an art into a science. Curr. Opin. Struc. Biol. **14,** 577–583
http://dx.doi.org/10.1016/j.sbi.2004.08.002
Ducruix A., Giegé R. (1999): Crystallization of Nucleic Acids and Proteins: A Practical Approach. (2nd Ed.), pp.7, IRL Press, Oxford Eds.
Durbin S. D., Feher G. (1996): Protein crystallization. Annu. Rev. Phys. Chem. **47,** 171–204
http://dx.doi.org/10.1146/annurev.physchem.47.1.171
Einsenhaber F. (1999): Hydrophobic regions on protein surfaces. Perspect. Drug. Disc. Des. **17,** 27–42
http://dx.doi.org/10.1023/A:1008766422140
Lafont S., Veesler S., Astier J. P., Boistelle R. (1994): Solubility, and prenucleation of aprotinin (BPTI) molecules in sodium chloride solution. J. Cryst. Growth **143,** 249–255
http://dx.doi.org/10.1016/0022-0248(94)90064-7
Lafont S., Veesler S., Astier J. P., Boistelle R. (1997): Comparison of solubilities and molecular interactions of BPTI molecules giving different polymorphs. J. Cryst. Growth **173,** 132–140
http://dx.doi.org/10.1016/S0022-0248(96)00834-2
Lee M. J., Kwon J. H, Shin J. S, Kim C. W. (2005): Microcrystallization of alpha-lactalbumin. J. Cryst. Growth **282,** 434–237
http://dx.doi.org/10.1016/j.jcrysgro.2005.05.012
McPherson A. (1999): Crystallization of Biological Macromolecules. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
McPherson A. (2004): Introduction to protein crystallization. Methods **34,** 254–265
http://dx.doi.org/10.1016/j.ymeth.2004.03.019
Ninomiya K., Yamamoto T., Oheda T., Sato K., Sazaki G., Matsuura Y. (2001): Morphology and solubility of multiple crystal forms of Taka-amylase A. J. Cryst. Growth **222,** 311–316
http://dx.doi.org/10.1016/S0022-0248(00)00912-X
Revalor E., Hammadi Z., Astier J. P., Grossier R., Garcia E., Hoff C., Furuta K., Okustu T., Morin R., Veesler S. (2010): Usual and unusual crystallization from solution. J. Cryst. Growth **312,** 939–946
http://dx.doi.org/10.1016/j.jcrysgro.2010.01.006
Rosenbaum D. F., Kulkami A., Ramakrishnan S., Zukoski C. F. (1999): Protein interactions and phase behavior: Sensitivity to the form of the pair potential. J. Chem. Phys. **111,** 9882–9890
http://dx.doi.org/10.1063/1.480328
Stewart L., Clark R., Behnke C. (2002): High-throughput crystallization and structure determination in drug discovery. Drug Discov. Today **7,** 187–196
http://dx.doi.org/10.1016/S1359-6446(01)02121-3
Srimahaprom W., Flood A. E. (2013): Crystal growth rates and optical resolution of DL-methionine hydrochloride by preferential crystallization from aqueous solution. J. Cryst. Growth **362,** 88–92
http://dx.doi.org/10.1016/j.jcrysgro.2011.10.056
Sleutel M., Willaert R., Gillespie C., Evrard C., Wyns L., Maes D. (2009): Kinetics and thermodynamics of glucose isomerase crystallization. Cryst. Growth Des. **9,** 497–504
http://dx.doi.org/10.1021/cg800756h
Suzuki Y., Sazaki G., Visuri K., Tamura K., Nakajima K., Yanagiya S. J. (2002): Significant decrease in the solubility of glucose isomerase crystals under high pressure. Cryst. Growth Des. **2,** 321–324
http://dx.doi.org/10.1021/cg025509f
Taulelle P., Sitja G., Pépe G., Garcia E., Hoff C., Veesler S. (2009): Measuring enthalpy of sublimation for active pharmaceutical ingredients: validate crystal energy and predict crystal habit. Cryst. Growth Des. **9,** 4706–4709
http://dx.doi.org/10.1021/cg900567z
Van Driessche A. E. S., Gavira J. A., Lopez L. D. P., Otalora F. (2009): Precise protein solubility determination by Laser confocal differential interference contrast microscopy. J. Cryst. Growth **311,** 3479–3484
http://dx.doi.org/10.1016/j.jcrysgro.2009.04.023
Van Holde K. E., Johnson W. C., Ho P. S. (2006): Principles of Physical Biochemistry. Pearson Prentice Hall, Upper Saddle River
Vekilov P. G., Feeling-Taylor A. R., Yau S. T., Petsev D. (2002): Solvent entropy contribution to the free energy of protein crystallization. Acta Crystallogr. D. **58,** 1611–1616
http://dx.doi.org/10.1107/S0907444902014312
Vekilov P. G. (2003): Solvent entropy effects in the formation of protein solid phases. Method. Enzymol. **368,** 84–105
http://dx.doi.org/10.1016/S0076-6879(03)68006-7
Visuri K., Kaipainen E., Kivimaki J., Niemi H., Leisola M., Polosaari S. (1990): A new method for protein crystallization using high pressure. Biotechnology (NY) **8,** 547–549
http://dx.doi.org/10.1038/nbt0690-547
Vuolanto A., Uotila S., Leisola M., Visuri K. (2003): Solubility and crystallization of xylose isomerase from Streptomyces rubiginosus. J. Cryst. Growth **257,** 403–411
http://dx.doi.org/10.1016/S0022-0248(03)01426-X

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