CDD: 547.75

THE INFLUENCE OF PRECIPITANT AND THAUMATIN CONCENTRATION ON THE PROTEIN AGGREGATION STATE AT LOW IONIC STRENGTHS

INFLUÊNCIA DAS CONCENTRAÇÕES DE PRECIPITANTE E TAUMATINA NO ESTADO DE AGREGAÇÃO DA PROTEÍNA A BAIXAS FORÇAS IÔNICAS

JORGE IULEK[1]
A. M. CARCAMO[2]
1 Professor do Departamento de Química da UEPG
2 Professor da Universidade Nacional Autónoma de México. Instituto de Química

ABSTRACT

Dynamic Light Scattering (DLS) studies were carried out to determine the aggregation state of thaumatin (a remarkable sweet tasting protein) in several solution conditions, involving changes on the precipitant (sodium and potassium tartrate) concentration, on the protein's own concentration and on the temperature. The measurements show that at lower precipitant concentration as well as at lower protein concentration, the aggregation is smaller. A higher temperature also helps in reducing the residual aggregation. Such data indicate that the search for better thaumatin crystals should be directed toward those conditions. Besides, there was no significant

alteration on the aggregation state at a given temperature value, even if the same value is reached after a subsequent elevation followed by a reduction (or vice versa), characterizing the aggregation phenomenon (which is little extensive) due to temperature as a reversible process.

Key words: dynamic light scattering; thaumatin; protein crystallization

1. Introduction

Protein crystallization is considered the modern bottleneck in protein three-dimensional structure determination (DUCRUIX and GIEGÉ, 1992). With the recent determination of the genomes of several organisms (including the human one), much attention has been directed to the question of determining the structure of the proteins that are encoded by the genes, which generally makes use of x-ray crystallography, dependent on the obtention of high quality protein crystals. The question of protein crystallization, up to then an almost trial-and-error enterprise, came up strongly to the scene, demanding the development of rational techniques which can direct the search for the adequate crystallization conditions. Among these, the Dynamic Light Scattering (DLS) technique has been shown to be the most useful one (D'ARCY, 1994) due to its capacity to indicate the conditions in which the protein appears most closely to a monodispersion solution, where the possibility of getting good crystals is enhanced. Several proteins, in different solution conditions, have been studied using this technique, and the results have been accumulating a basic knowledge which helps to interpret the forces which come into action when a protein crystallizes. In this article, the influence of two variables, the ionic strength and the protein concentration, on the thaumatin (from *Thaumatococcus daniellii*) aggregation state in solution were studied, so that better conditions for its crystallization could be determined. Thaumatin (207 aminoacid residues, MW = 22,000 Da) is a well-known protein which has the exceptional property of presenting a highly sweet taste (GREEN, 1999). Its aggregation properties in a high ionic strength zone have already been reported (JUÁREZ-MATÍNEZ et al., in press). The results when the protein concentration as well as the ionic strength are varied at a low ionic

strength zone, are presented here.

2. Materials and Methods

All reagents used were of analytical grade. Thaumatin was purchased from Sigma Chemical Co.. The water used to prepare the solutions and their dilutions was of the milli-Q (Millipore Corporation) type.

2. 1. Variable Protein Concentration

2. 1. 1. Conditions:

- Constants: buffer type (K/Na phosphate, 0.1 mM, pH 7.0), precipitant agent concentration (7.5 % (w/V)];

- Variables: protein concentration (from 10 to 20 mg/mL, at 2 mg/mL steps) and temperature (4 and 18 ºC).

2. 1. 2. Stock solutions:

- Na/K phosphate buffer 100 mM, pH 7.0;

- 15 % (w/V) Na/K tartrate solution, prepared in the above cited buffer;

- 40 mg/mL thaumatin solution, prepared in the above cited buffer.

2. 1. 3. Sample preparation:

Mixtures were made extemporaneously. Right after that, they were put into the apparatus to measure the data.

2. 1. 4. Measurements

The aggregation state of thaumatin was evaluated at two temperatures, 4 °C and 18 °C (typical temperatures for making protein crystallization assays), in a forward and reverse fashion (first round, 4 °C, then 18 °C; second round, the same sample cooled back to 4°C and then heated again to 18 °C), making 15 measurements of DLS data at each temperature and at each round, using a DynaPro 801TC equipment (Protein Solutions Inc.). The software DYNAMICS (Protein Solutions Inc.) was used later on to calculate the respective values of the hydrodynamics Radius (R_h) and to estimate the molecular weight (MW) of the particles in solution.

2. 2. Variable Precipitant Agent Concentration

2. 2. 1. Conditions:

- Constants: buffer type (phosphate K/Na, 0.05 mM, pH 7.0), thaumatin concentration (20 mg / mL);

- Variables: precipitant concentration, tartrate [from 2.5 to 7.5 % (w/V), at 1 % (w/V) steps] and temperature (4 and 18 °C).

Stock solutions, sample preparation and measurements were made just like in item 2.1.

3. Results and discussion

3. 1. Variable Protein concentration

Table I shows the compositions of the various samples assayed and the respective average hydrodynamic radius (Rh); figure 1 relate those to the estimated molecular weight (MW).

TABLE I – Sample mixtures and compositions to assay the influence of thaumatin concentration on its aggregation state in solution.

Final Thaumatin Concentration / [mg/mL]	[Volume of Thaumatin Stock Solution at 40 mg/mL] / μL	[Volume of Phosphate Buffer at 100 mM] / μL	[Volume of Na/K Tartrate Solution at 15 % (w/V] / μL	R_h / nm, First Round, 4 ºC	R_h / nm, First Round, 18 ºC	R_h / nm, Second Round, 4 ºC	R_h / nm, Second Round, 18 ºC
10	125	125	250	2.77	2.70	2.78	2.69
12	150	100	250	2.86	2.82	2.89	2.82
14	175	75	250	2.92	2.85	2.90	2.82
16	200	50	250	2.99	2.90	2.96	2.88
18	225	25	250	2.97	2.87	2.99	2.87
20	250	0	250	3.03	2.93	3.04	2.94

Figure 1 shows that an increase in the thaumatin concentration leads to some aggregation of the macromolecules in solution, but this is not very extensive and there is a prevalence of the dimmer form. Besides, a lower temperature also leads to this aggregation, although the process is reversible.

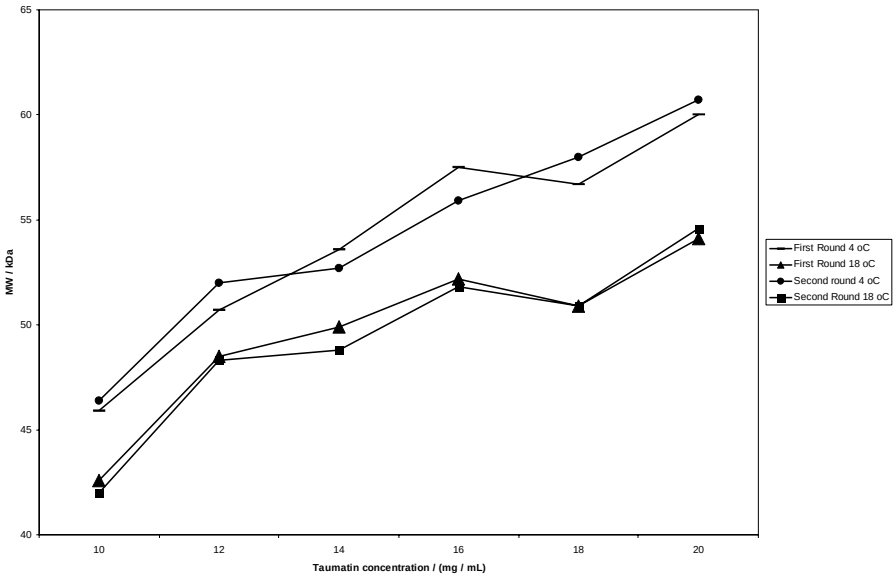

Figure 1. Influence of thaumatin concentration on its aggregation state, measured at 4 and 18 °C (forward and reverse).

3. 2. Variable Precipitant Agent Concentration

Table II shows the compositions of the various samples assayed and the respective average hydrodynamic radius (Rh); figure 2 relates those to the estimated molecular weight (MW).

Figure 2 shows that an increase in the precipitant concentration leads to some aggregation of the macromolecules in solution, and again, this is not very extensive and there is prevalence of the dimmer form also in these conditions. Besides, as was observed for the protein at several different conditions, a lower temperature also leads to this aggregation, although the data show this process is reversible as well.

TABLE II - Sample mixtures and compositions to assay the influence of precipitant concentration on thaumatin aggregation state in solution.

Final Thaumatin Concentration / [mg/mL]	[Volume of Thaumatin Stock Solution at 40 mg/mL] / μL	[Volume of Phosphate Buffer at 100 mM] / μL	[Volume of Na/K Tartrate Solution at 15 % (w/V] / μL	R_h / nm, First Round, 4 ºC	R_h / nm, First Round, 18 ºC	R_h / nm, Second Round, 4 ºC	R_h / nm, Second Round, 18 ºC
20	375	250	125	2.77	2.70	2.78	2.69
20	375	200	175	2.86	2.82	2.89	2.82
20	375	150	225	2.92	2.85	2.90	2.82
20	375	100	275	2.99	2.90	2.96	2.88
20	375	50	325	2.97	2.87	2.99	2.87
20	375	0	375	3.03	2.93	3.04	2.94

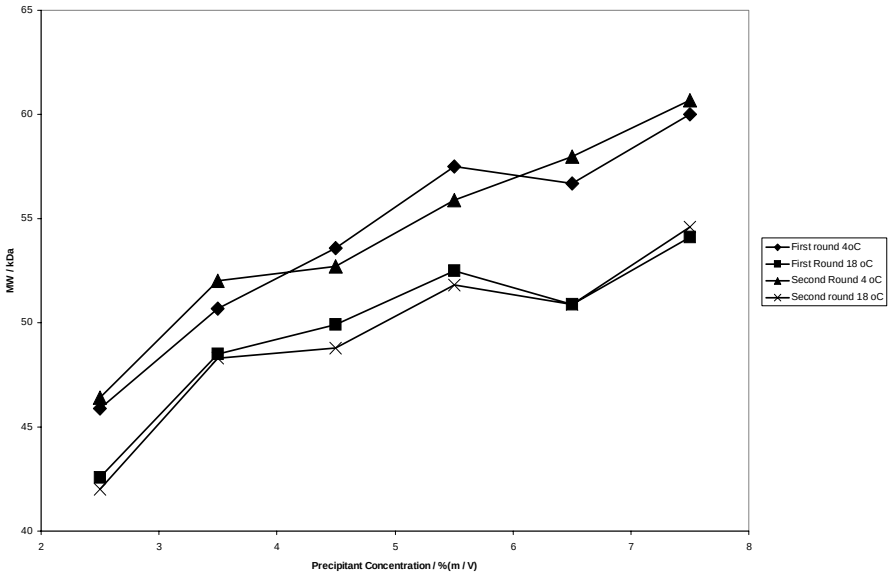

Figure 2. Influence of the precipitant concentration on the aggregation state of thaumatin, measured at 4 and 18 °C (forward and reverse).

h / m, cond und, °C
2.69
2.82
2.82
2.88
2.87
2.94

5. Conclusions

Dynamic Light Scattering measurements showed that the usage of lower thaumatin concentrations and lower ionic (sodium and potassium tartrate) strength can also lead to good protein crystals, in addition to the crystallization conditions that have been commonly used, which use high ionic strengths (KO et al., 1994). Also, to crystallize the protein, the variable temperature can be explored, yet it was observed that lower temperatures tend to make the protein aggregate, this one normally being a step, at a slow rate, involved during protein crystallization. More experiments must now be carried out to explore the influence of other variables on the crystallizability of this model protein, thaumatin.

RESUMO

Estudos por Espalhamento Dinâmico de Luz (DLS) foram realizados a fim de determinar o estado de agregação da taumatina (uma notável proteína com gosto doce) em várias condições de solução, envolvendo mudanças na concentração do precipitante (tartarato de sódio e potássio), na concentração da própria proteína e na temperatura. Os resultados mostram que em baixa concentração do precipitante, assim como em baixa concentração de proteína, a agregação é menor. Uma temperatura maior também ajuda a diminuir a agregação residual. Tais dados indicam que a busca por melhores cristais de taumatina deveria ser direcionada para essas condições. Além disso, não houve alteração significativa no estado de agregação num dado valor de temperatura, mesmo quando tal valor era alcançado após uma subseqüente elevação seguida de redução (ou vice-versa), caracterizando o fenômeno de agregação (que é de pequena extensão) devido à temperatura como um processo reversível.

Palavras-chave: espalhamento dinâmico de luz; taumatina; cristalização de proteínas

Acknowledgements

The authors thank the Third World Academy of Sciences for the South-South Fellowship Grant and the Instituto de Química from UNAM for the financial support, which allowed the travel and staying of Prof. Jorge Iulek in Mexico where this study was carried out.

Recebido para publicação em 1/10/2000.
Aceito para publicação em 1/12/2001.

Endereço para contato: iulek@uepg.br

REFERENCES

1 D'ARCY, A. Crystallizing proteins: a rational approach. **Acta Cryst.**, v. D 50, p. 467-471, 1994.

2 DUCRUIX, A.; GIEGÉ, R. **Crystallization of Nucleic Acids and Proteins**. New York : Oxford University Press, 1992.

3 GREEN, C. Thaumatin: a natural flavour ingredient. **World Rev. Nutr. Diet.**, v. 85, p. 129-132, 1999.

4 JUÁREZ-MATÍNEZ, G.; GARZA, C.; CASTILLO, R.; BAIRD, J. K.; MORENO, A. A dynamic light scattering investigation of the nucleation and growth of thaumatin crystals. **Journal of Cryst. Growth**, in press.

5 KO, T. P.; DAY, J.; GREENWOOD, A.; McPHERSON, A. Structures of three crystal forms of the sweet protein thaumatin. **Acta Cryst.**, v. D 50, p. 813- 825, 1994.