

A same plasmatic protein has own autonomy depending of temperatures and pH values

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The same protein has coagulant or fibrinolytic activity depending on the pH or temperature value. The protein has coagulant action in low pH and temperature or fibrinolytic activity in high pH and temperature. A same protein has own autonomy to develop coagulant or lytic activity, depending of its environment. Many diseases may be explained by changes in the internal environments as pH and temperatures affecting the structure native of proteins. The biological activity of proteins depends on its three –dimensional structured which is derived by the folding of the linear amino acid polymer synthesized on ribosome. Since the magnitude of these forces is influenced by environmental conditions, a protein stability depends of factors that temperature, pressure, pH, extraneous agents and ionic strength. The fold of Proteins is subject its environment. The structural plasticity is necessary for the catalytic activities of ordered proteins, and the amino acid drive different responses to changes in their environment. The one that with little difference in the range of pH and temperature, a same protein can behave of different function without becoming denaturalized would help to understand the adaptation that undergoes these macromolecules to the environment changes.

Keywords: Protein, pH, coagulation, environments, temperature.

INTRODUCTION

The biological activity of proteins depends on its three – dimensional structured which is derived by the folding of the linear amino acid polymer synthesized on ribosome. Protein folding is generally a fast reversible reaction. The physical forces that determine the fate of unfolding – refolding reactions include, but are not limited to, the hydrophobic, the van der Waals, electrostatic interactions, hydrogen bonds and covalent cross- link. Since the magnitude of these forces is influenced by environmental conditions, a protein stability depends of factors that temperature, pressure, pH, extraneous agents and ionic strength. The biggest challenge of a modern protein chemist is therefore, is to determinate the contributions of individual amino acid to the stability of a protein as a functions of environments conditions. (Khan 1990) Atoms and molecules constitute matter and living beings serving

vital functions in the form of macromolecules that give energy as carbohydrates carbon and lipids or DNA formed by desoxirribonucleotides with information to express the protein synthesis through the 20 different amino acids, which are encoded by a triplet of bases contained in the mRNA. Proteins synthesized according to its conformation have a vital function in living things. May de proteins after synthesis under determinates conditions have opposing functions without losing its biological functions? (Roder et al., 2006; Apetri et al., 2006; Riley et al., 2007; Maki et al., Abel et al.,)

Proteins take after its synthesis a characteristic three-dimensional conformation depending on the primary structure of the same. This conformation can be altered by changes in their environment regulated by the temperature and pH (Serdyuk 2007). In proteins may be happened for different reasons such as human disease, forms denaturalized by acid pH or high temperatures, microorganism disorders in their three-dimensional conformation, altering its biological activity that may be

permanent or not in certain favorable circumstances (Uversky 2010).

Proteins possess functional differences according to its three-dimensional conformation and the different internal folding is based on the type of amino acid and the same complexity. In less structured proteins which are feasible to generate more changes in its fold, depending on the areas known as random or Alpha Helix, which makes them more susceptible to changes depending on the environment that surrounds them at different situation. The aromatic amino acids can influence the internal displacement of protein and this is crucial at the time of their biological role

(Wright and Dyson 1999; Fink 2005; Dunker et al., 2002). Under physiological changes less structured proteins are the most they can adapt to the variation of pH and temperature. ((Bemporad et al., 2008)

In relation to certain properties of proteins as enzymatic activity (catalytic site), the solubility depending on its environment place, stability, etc., are fundamental different amino acids and its location within the polypeptide chain. Therefore the values of pK and pHi for proteins depend on such residues and ionization of its. There are experimental models of biological systems that can predict the behavior of proteins. (Pace et al., 2009)

As part of the requirements that are required so that a protein acquires its biological function is its three-dimensional conformation. To do so must coexist different structures in an organized and coordinated manner. But it is that there are regions which are called randomized (sequence of amino acids without a predetermined order), which would allow proteins adapted to complex situations and to maintain their native biological activity or perhaps same disorganization enable new functions as part of the alterations which usually occur in the environments (Boehr and Wright 2008)

Of all the roles the proteins, enzyme activity perhaps the least compatible with the possible disordered in the three-dimensional conformation present in certain sites of the polypeptide chain. But a news research have revealed that a strict order to those protein catalytic functions is not always necessary (Tokuriki and Tawfik 2009; Dyson and Wright 2005; Tompa 2005).

The relationship between activity of proteins and their three-dimensional conformation with their environment can be perfectly analyzed in aqueous solutions of pure protein. The structural plasticity is necessary for correct function of proteins, and the amino acid drive different responses depending the situations its have. This study we theorized as a same protein may according to different pH and temperature with which it precipitate, have opposing functions. That was shown with a protein how varies its activity with a pH in the range from 8 to 9, and temperature either 25 °C or 4 °C, behaves owns coagulant function or otherwise induces lysis of fibrin

respectively. However, important questions remain to be answered concerning the structural properties of proteins during early stages of folding. It is very important to establish a barrier between which it subsequent to express the genetic code and the autonomy in the activities of proteins after its synthesis. The one that with little difference in the range of pH and temperature, a same protein can behave of different function without becoming denaturalized would help to understand the adaptation that undergoes these macromolecules to the environment changes.

MATERIALS AND METHODS

Protein was anyisolated of human plasma (blood center fundation, Cordoba Argentina). pH measurement was carried out with a pH meter Hanna Instruments (USA), calibrated with buffer phosphates pH 4 and pH 7. Buffer used to salting in and salting out was sodium Phosphate pH 9 150 mM. The protein fraction was precipitate with trichloroacetic 0.1 %. The salting out was carried out in the range of pH 8 to 9 and the activity of each one of them was determined. The coagulation activity was assay with fibrinogen reagent test (Multifibren U, Dade Behring, Lot 519847 A, Germany) and fibrin plate method to performed lysis respectively. The protein fraction were precipitated either 25 °C or 4°C and assay de clot and lysis test after each temperature. The fibrin plate was performed adding 3 ml fibrinogen free of plasminogen solution was clotted in a glass dish 15 cm diameter by mixing with 0.5ml of thrombin (Stago, USA) in 0.75 ml 0.02 M sodium borate buffer, pH 8.8. Native polyacrylamide gel electrophoresis was performed to run and compare the same protein in different temperature and pH precipitation conditions. Native 10% of polyacrilamide was prepared with Acrylamide and N'N'N'N' Bis-acrylamide (29 g/100 ml and 0.8 g/100 ml respectively). Gel buffer pH 8.8 (Tris – HC) and running buffer pH 8.9 (Glycine – Tris). The electrophoretic run was performed diuring 40 min at 120 volts. Protein was stained and destained with a standard Silver stain method

RESULTS

Keeping constant same variables except the pH and temperature the salting out was performed. First the relationship between the pH which the protein precipitation happened and the resulting activity of it was assaied. As we can see the coagulant activity of precipitate protein increases an initial pH of 8 until pH of 8.5. In contrast the lytic activity of the same protein increases from a pH 8.6 until pH 9. (Figure 1). Following with the precipitation of a protein, it was



Figure 1 Native electrophoresis shows the same protein. Line 1 with fibrinolytic activity and line 2 with coagulation activity

performed first at low temperature (4 °C), during two hours. After this, the coagulation activity was confirmed. On the other side in similar conditions of buffer and precipitant solution another precipitation carried out at 25 °C, during 12 hs. Lysis activity happened at this temperature; without denaturation in both cases. (Figure 2) The electrophoretic migration of this protein, in 10 % polyacrilamide shown one component with the same electrophoretic mobility. These proteins bands corresponds for both pH 8 to 8.5 (lane 1) with coagulation results and 8.6 to 9 (lane 2) with lityc activity. precipitation and 4 °C (lane 1) and 25 °C (lane 2) Respectively. There is no doubt that this is the same protein, but are different in terms of the activity of the same according to the environments conditions in which was precipitated. (Figure 3)

DISCUSSION

While the three-dimensional conformation is present and required for all types of proteins, the Globular proteins are where the more it affects or influence changes that

can experience the different structures. We must also remember that the folding in Globular proteins are conditioned by the internal hydrophobic environment of the chain and the external solvation layer. Any change in temperature can promote its denaturation or contrary stability. There are proteins under specific solutions conditions that the temperature changes do not introduce denaturations effects. In this research the same plasmatic protein at different temperatures take a 3D structure whit out loss its biological activity. Both 4 °C and 25 °C in determinates conditions of buffers solution, have coagulation or fibrinolytic activity respectively. Researches in this field try to explain that intrinsic mechanisms occur in proteins with different temperatures. So far the knowledge about the location of some amino acids in the polypeptide chain, could explain these phenomena of changes in the activity of proteins without denaturation of the same. The environment is that provides the values of pH and in some cases the presence of proteases that cut in specific sites of the amino acid chain. It may therefore be that it can precipitate a same protein, without denaturalization and at the same time to have opposing functions to different

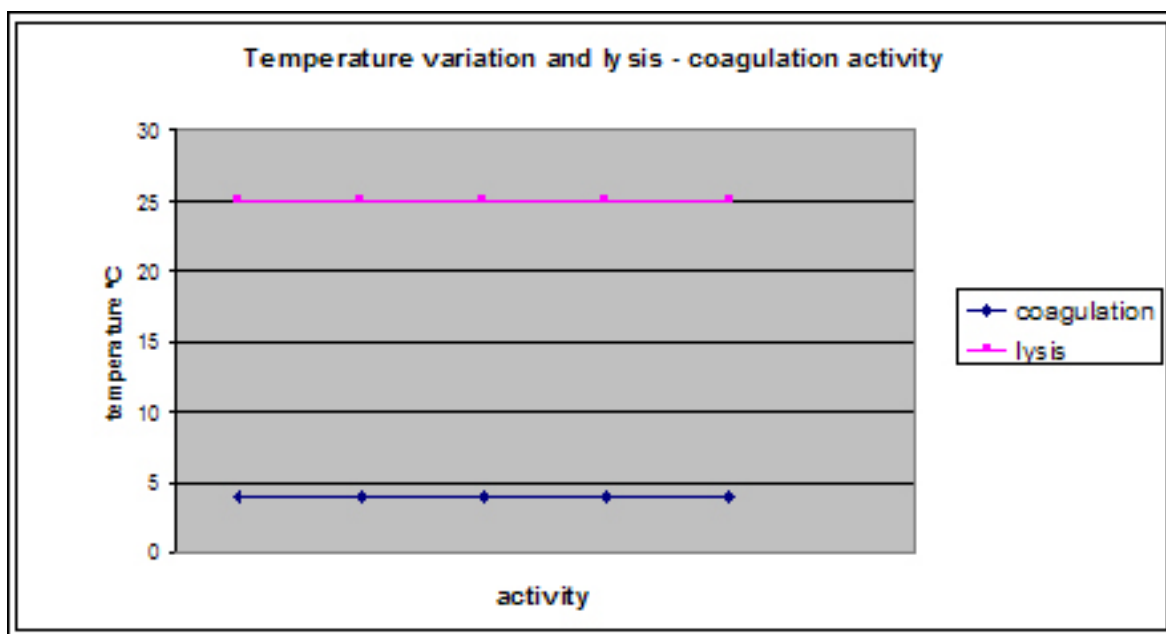


Figure 2 Temperature variation and lysis- coagulation activity

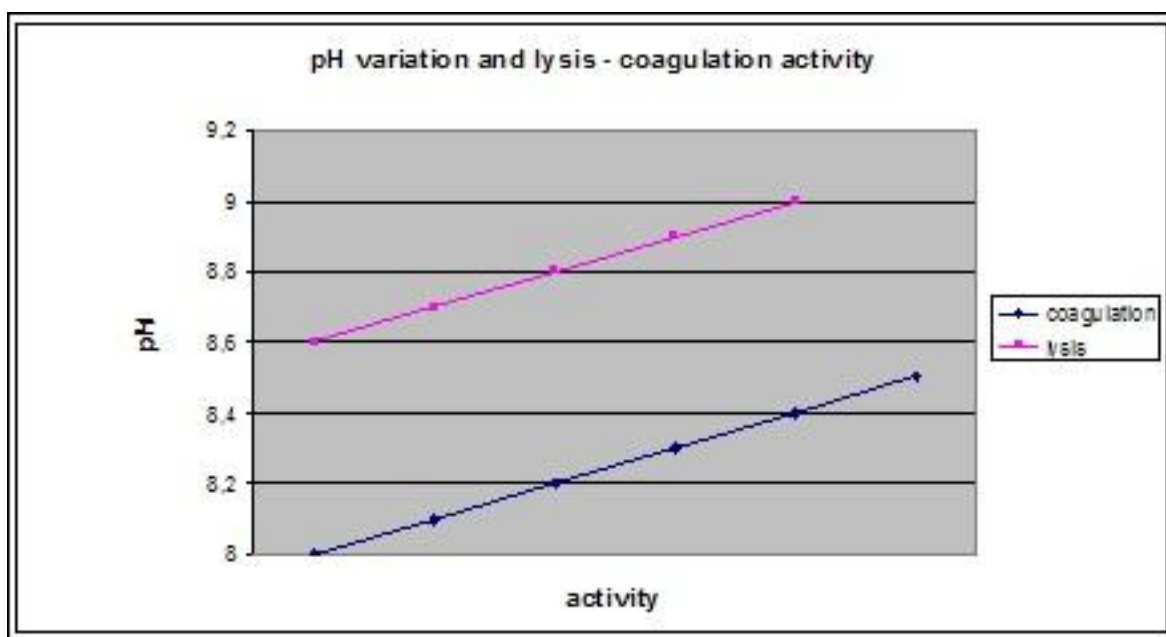


Figure 3 pH Variation and lysis- coagulation activity

pH or temperature. The protein in study has an isoelectric point at pH either 8 and 9 in which is not denatured with both biological functions.

When we refer to the methods of isolation of proteins, the isoelectric precipitation is the most safe and effective. In the case of plasmatic proteins most of them has your pHi between 4 and 6, so above the same proteins have in a negative charge. It is very important to know the pHi for

proteins and if is a pH where biological activity is not altered. In this way there are extracellular proteins where the environments conditions are different that in intracellular medium. The pH variations outside of the cells are more drastics, but It is more easy to see such opposite (coagulation – lysis) reactions as in the case of this protein, despite being more exposed to changes in the internal environments. The proteins in the extra-

cellular compartments have better browsing movement, can therefore possess more transition and autonomy in their functions.

The isolation of protein is usually the first step to its purification. Here find the method to obtain the protein in stable conditions and avoid its denaturation is a stage that must standardize. Normally usually carry out cold (e.g. solvents), but also it can be carry out at room temperature taking control over those factors that can make you lose its biological activity. In this case this protein has the property to precipitate with out denature at 25 °C. In the other side if the temperature downs to 4 °C it to precipitate too but with another biological function.

CONCLUSION

While the structure and function of proteins depends on the information that this deposited in the genetic code, after its synthesis they may found situations that have nothing to do with its origin. This change in the environment specifically at the extra cellular level makes the proteins to adapt, and these process can happened events as studied in this research, which to showed that a same protein can behave opposite with out denatured, due to changes such as pH and temperature. They have own autonomy after its synthesis. May be the key in the future to diagnostics and treatment of specific diseases.

ACKNOWLEDGEMENT

Dr Carrizo Luis H and all the staff of the central Blood Bank Foundation.

REFERENCES

- (in press) 6573–6582. Bemporad F et al., (2008). Biological function in a non-native partially folded state of a protein. *EMBO Journal* 27, 1525
- Abel CJ, Goldbeck RA, Latypov RF, Roder H, Kliger DS ? provide date Conformational equilibration time of unfolded protein chains and the folding speed limit. *Biochemist.*
- Apetri AC, Maki K, Roder H, Surewicz WK (2006). Early intermediate in human prion protein folding as evidenced by ultrarapid mixing experiments. *J. Am. Chem. Soc.* 128:11673-11678.
- Biol Chem. ;284:13285–13289.
- Boehr DD, Wright PE (2008). *Biochemistry. How do proteins interact?* Sci. 320: 1429–1430.
- Dunker AK, Brown CJ, Lawson JD, Iakoucheva LM, Obradovic Z (2002). Intrinsic disorder and protein function. *Biochemistry* 41: 197–208.
- Dyson HJ, Wright PE (2005). Intrinsically unstructured proteins and their functions. *Nat. Rev. Mol. Cell. Biol.*, 6:197-208.
- Fink AL (2005). Natively unfolded proteins. *Curr Opin Struct Biol* 15: 35–41.
- Maki K, Cheng H, Dolgikh DA, Roder H, ? provide date Folding kinetics of staphylococcal nuclease studied by tryptophan engineering and rapid mixing methods. *J. Mol. Biol.* (in press).
- Pace CN, Grimsley GR, Scholtz JM (2009). Protein Ionizable Groups: pK Values and Their Contribution to Protein Stability and Solubility. *J. Biol. Chem.* 284:13285–13289.
- Riley PW, Cheng H, Samuel D, Roder H, Walsh P, Dimer N (2007) dissociation and unfolding mechanism of coagulation factor XI apple 4 domain: Spectroscopic and mutational analysis. *J. Mol. Biol.* 367:558-573.
- Roder H, Maki K, Cheng H (2006). Early events in protein folding explored by rapid mixing methods. *Chem. Rev* 106:1836-1861.
- Serdyuk IN (2007). Structured proteins and proteins with intrinsic disorder. *Molecular Biology*, 41:262-277.
- Tokuriki N, Tawfik DS (2009). Protein dynamism and evolvability. *Science*, 324:203-207.
- Tomba P (2005). The interplay between structure and function in intrinsically unstructured proteins. *FEBS Lett*, 579:3346-3354.
- Uversky VN (2010). The mysterious unfoldome: structureless, underappreciated, yet vital part of any given proteome. *J. biomed. Biotechnol.* 2010:568068.
- Wright PE, Dyson HJ (1999). Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *J. Mol. Biol.* 293: 321–331.
- Yahiya Khan M (10 August 1990). Protein folding revisited. *Current science*, vol. 59, NO 15.