Conformational Transition of the Intermolecular Interaction of Protein System of Buffalo UHT Milk

Ali Ibrahim Ali Mansour

Dairy Science Department, Faculty of Agric., Al-Azhar University, Assiut, Egypt

Abstract

The reversible casein/whey protein complex formation in UHT milk was described by equilibrium between two main conformations of the protein, which is follo¬wed by the change of the proton-binding properties as a function of pH. Thermodynamics and some kinetic pro¬perties of pH-depending phase transition of milk prot¬ein system were discussed. The kinetic measurements supported the intermolecular and cooperative "all-or-none" change of casein micel¬les caused by the action of protons. The results of this studies suggested that, the metastability of conformation is predominantly due to the energy barriers, preventing the equilibrium transition of the protonated milk protein system above a critical pH value. The overall equilibrium constant of the pro¬cess depended on the heating strength and the pH value of milk.

Key words: Dairy, Heat stability, Heat treatment, Kinetics, UHT milk, Protein system

Introduction

Some proteins and nucleic acids are known to exhibit thermodynamically metastable conformational states and non-equilibrium transitions in the system in their pH titrations in the acid range (Neumann and Katchalsky 1975, Pohl 1972 as well as Spodheim and Neuman 1975). The phase transition has been studied by potentiometric and spectophotometric titrations and the kinetics of the transition by means of the pH or temperature jump re¬laxation technique (Pohl 1969, Revzin et al. 1975 and Mansour 2008). The existence of hysteresis in the titration behaviour of milk proteins has led us to delve into the structures and the kinetic barriers that exist in the molecules at the various stages of the titration cycle. The structural domain involved in these changes is the casein micelle. By heating, the surfaces of the micelles are covered successively with serum proteins. In order to derive the general properties of this complex binding mechanism it is useful to discuss the standard enthalpies and entropies.

Under isothermal-isobaric conditions, every equilibrium state of casein system is completely determined by the two parameters (pH) and degree of protonation (α).

However, in the domain of hysteresis a complete des¬cription of the actual state is obtained in addition to knowledge of the history of the system (Kirchmeier 1979).

Some of the kinetic and thermodynamic results of the structural changes between the two conformational states of the protein system using a number of ultra high temperature heating treatments will be investi¬gated and discussed which might lead to a quantitative description of the observed effects.

Materials and Methods

Heat treatment. Bulk milk samples used in the present study were obtained from the fresh morning bulk buffalo's milk of herd of Mostorod Experimental station. Whole milk was heated after defatting by centrifugation at 5000 XG for 20 min at 20°C. Samples of UHT milk were obtained by heating in tightly closed glass tubes in an oil-path with shaking. The milk was preheated to 80°C and then rapidly heated to 130°C for 5, 10, 15 and 20 min. After cooling at room temperature the samples were analyzed.

Titration method. The acid-base titration data were obtained using an automatic recording potentiometric titration appara¬tus, Metrohm AG, Herisau, Switzerland. Potentiometric titration of milk was carried out by the following procedure (Kirchmeier 1977): Ten ml milk sample was titrated from pH 7.0 to pH 5.0 in 0.2 pH-intervals, using 0.01 N HCl, with constant stirring-speed. The equilibrium was fixed at 40 sec for each pH-interval.

---

* Corresponding author E-mail dr_bahbo@yahoo.com

After reaching to pH 5.0, the back titration to pH 7.0 was occurred using 0.01 N NaOH under the same conditions

The results of potentiometric titrations are plotted in terms of protonation degree ( ) as a function of pH. The mean degree of protonation ` is defined as the ratio between the num¬ber of moles of bound protons [HA] and the number of moles of protonation partners [HA] + [A-].

Determination of soluble casein. The soluble casein was determined in each titration interval (0.2 pH) by Kjeldahl method as modified by Rowland 1958.

Results and Discussions

The reversible formation of β-laotoglobulin-κ-casein complex during the ultra high temperature heating can be described by equilibrium between two main con-formations of the protein, which can be followed by the change of protonation degree (α), transition degree (θ) or the cooperativity (δ).

The decrease in pH of milk from 7.0 to about 5.0 re¬sults in protonation of casein micelles, colloidal calcium phosphate and whey proteins, which are the predominant protonation partners in this pH range. This protonation is indicated by the increase in α-value from zero to one (Fig. 1). The acid titration curve of milk α (pH) can be des¬cribed by: pH = pKa + log (1 - α)/ α

Where: pKa is an apparent pK value, α is the mean degree of a protonation.

This acid branch of titration curve corresponds to the metastable protonation equilibrium:

Casein.coll.CaPhos. + H+ → H-Gasein + sol.CaPhos.
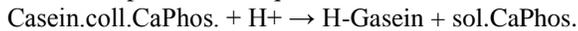
Experimentally, the results of nitrogen determination in the uncentrifugable part during the acid titration of milk illustrate a cooperative conformation changes by the casein.

n (H-Casein)   ↔   (H-Casein) n

       Disaggregation   Aggregation

It can be seen that only two states exist in equilib¬rium, A: n (H-casein) in solution, and B: (H-casein) n as an aggregate, whereas the concentrations of all intermediates are negligible. This all-or-none behav¬iour reflects the high cooperatively of the process.
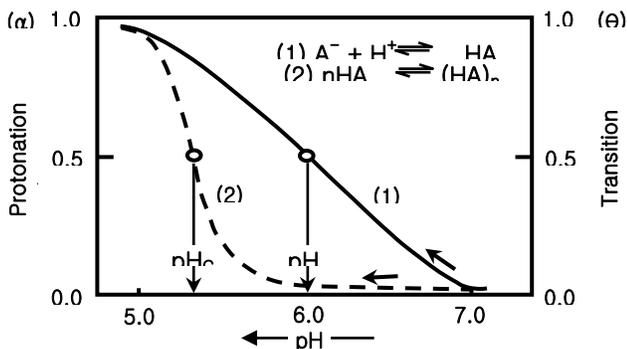
Fig. 1. The pH-dependence of the overall degree of protonation (α) and phase transition (θ) of milk proteins.

It is interesting to calculate the degree of conformational transition (θ) as a function of pH values according to:

Θ = (Aggregation) / [(Aggregation) + (Disaggregation)]

From Figure 1 (transition-pH relation) the pH at which = 1/2 is termed the critical pH value (pHC). In unheated milk, it is found that pH = 5.4. This means that, 50% of casein exists in dissociated form and the other 50% in aggregation state.

In conclusion, under the conditions of the potentiometric acid-base titration method, the cooperative conformation changes and thermodynamic metastability appear on the resolution of the casein micelle caused by the action of protons:
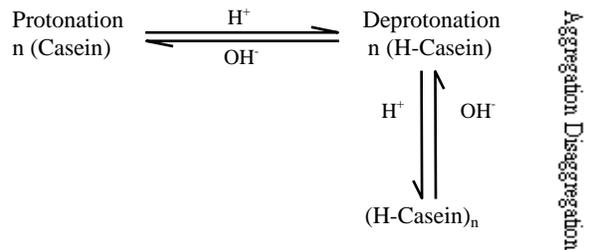
More direct information about the conformational changes of milk proteins during titration can be derived from the potentiometric data. As seen in the back titration from pH 5.0 to 7.0 with NaOH (Figure 2), a different titration curve is obtained. It can be showed that, the acid titration curve and the base curve form a hysteresis loop. The values of (dpH), that is the deg-ree of hysteresis area comprised between the titration curves are also calculated (Table 1).
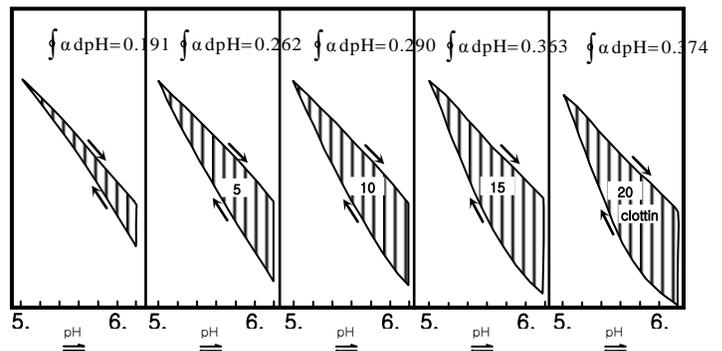
Fig. 2. Potentiometric acid-base titration of UHT milk heated at 130°C for 5, 10, 15 and 20 min (heat-coagulation state) compared with raw milk (Integral curves)

It appears from Figure 2 that, the size of hysteresis area is greater in ultra temperature heated milk than in un-heated milk and increased for longer heating time. These results agreed with the recent findings (Kirchmeier, 1977). The result¬ing expansion of the hysteresis loop is attributed to the progressive covering of the casein micelles with serum proteins as a result of heating (El-Shobery 1983).
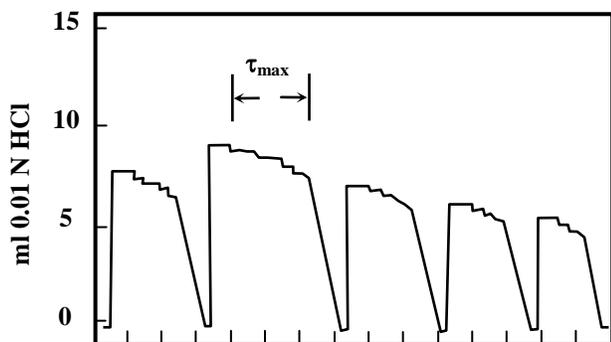
Fig. 3. Part of recording diagram of titration processing of UHT milk as a model of relaxation time. The time until the equilibrium state after changing the end point potentials 0.2 pH unit is the relaxation time (τ).

Furthermore, it is found that pKa values are shifted toward lower pH for higher UHT-heat treatments (Table 1). It thus seems that in metastable milk protein system an electrostatic energy barrier, which prevents the aggregate formation, can be passed if the degree of protonation reaches a critical value (pHC).

Table 1. Effect of heating time in UHT-heating process on some physicochemical parameters in hysteresis range of milk by potentiometric acid-base titration

Heat treatment	$\oint \alpha$ dpH *	pKa	τ_{max} (min)
Raw milk (25°C)	0.191	5.84	5.5
130°C / 5 min	0.262	5.54	7.0
130°C / 10 min	0.290	5.50	8.8
130°C / 15 min	0.363	5.44	11.0
130°C / 20 min	0.374	5.40	11.0

$$* \oint \alpha\,dpH = \frac{\text{hysteresisloop(mg)}}{\text{area unit}[1\,\alpha\,x\,1\,pH](mg)} \quad \text{(El-Shobery 1983)}$$

It is worthy of note that, the hysteresis can be explai¬ned as a consequence of the entropy changes of the system. In other words, the hysteresis loop, obtained from the isothermal-isobaric potentiometric acid-base titrations is a direct measure of free enthalpy ΔG and entropy production ΔS of system (Neumann 1973).

The product $-T\Delta S$ as a function of the loss of free energy of hysteresis-circulation can be calculated from:

$(\Delta G)p.T. = -T (\Delta S)p.T.$

Geometrically, the closed area of hysteresis corresponds to a cyclic integral α dpH, which is experimentally measurable. This integral α dpH is associated with an entropy increase and a loss of free enthalpy through¬out the following equations:

$$\Delta G° = -2.3\,RT \oint \alpha\,dpH$$

$$\Delta S° = +2.3\,R \oint \alpha\,dpH$$

Where: R is the gas constant (8.314 J/ mol . K) and T is the absolute tem¬perature (temperature C + 273).

For the Thermodynamic analysis of the potentiometric hysteresis, it is necessary to calculate the free en¬ergy, which is used for the restoration of the metastable states.

The experimental results of the energy calculations are presented in Table 2. It is seen that:

$\Delta G° (25°C) = -1088.3$ J / mol

$\Delta S° (25°C) = +3.652$ J / mol . degree

By UHT heating, the surfaces of casein micelles are covered successively with serum proteins. Due to this coating additional energy barriers are obtained. It is found that, the energy of entropy ($\Delta S°$) in the case of 130 C / 5 min heated milk samples is 5.010 J / mole and increased gradually with increasing of heating time. The contact between the two proteins (casein and whey proteins) may result in a large binding enthalpy. This association system contains the enthalpy and entropy terms of hydrogen bonding, hydrophobic interactions, etc.

Table 2. The energetic changes associated with differ¬ent UHT heating time in hysteresis range of milk

Heat treatment	Free enthalpy ($\Delta G°$) J / mol	Entropy ($\Delta S°$) J / mol . degree
Raw milk (25°C)	- 1088.3	5.84
130°C / 5 min	- 2019.0	5.54
130°C / 10 min	- 2234.6	5.50
130°C / 15 min	- 2797.2	5.44
130°C / 20 min	- 2882.3	5.40

In order to calculate the rate constants of transition reaction, the following relationship can be used (Kirchmeier and El-Shobery 1981):

$$\oint \alpha\,dpH = \log 4\,\tau_{max}\,kf$$

Then "the equilibrium constant of the all process can be expressed as (Pohl 1972):

$K = kf / kb$; $1 / \tau = kf + kb$

Where: kf is the velocity constant of the acid titration (forward k); kb is the velocity constant of the base titration (backward k) and K is the thermodynamic equilibrium constant of phase transition. In this study, it depends on pH, temperature and ionic strength.

Table 3. Some kinetic parameters of milk protein system by acid-base titration process

Heat treatment	pHC	Kf x10^{-2}	Kb x10^{-2}	K	δ
Raw milk (25°C)	5.22	7.1	11.1	0.64	0.64
130°C / 5 min	5.20	6.5	7.8	0.83	0.55
130°C / 10 min	5.17	5.5	5.9	0.93	0.52
130°C / 15 min	5.10	5.2	3.9	1.33	0.44
130°C / 20 min	5.00	5.4	3.7	1.46	0.42

It is interesting to note that, cooperativity is deter¬mined by the product of (δ) (Schwarz and Engel 1972), where:

$$\tau_{max} = \frac{1}{4\,\delta\,k_f}$$

The smaller δ (≤ 1) means more all-or-none behaviour. By $\delta = 1$ no cooperativity. The results are noted in Table 3. The constant of the regarded aggregation / disaggregation transition is considered K. The kinetic properties of such systems are described in terms of the relaxation time measurements, the relaxation time (τ) is de¬fined as the required time for reaching the equilibrium state of the system after each small perturbation with the very small pH interval changes. It is observed that, the τ max is located in the middle point of transition by $\theta = 0.5$. Under the recent conditions, it is found that, the equilibrium of the reversible aggregation process of milk protein system is not hold true. The thermodynamic results noted in Table 3 reveal that, the equilibrium constant "K" of the cooperative process is usually quite high. These kinetic data are consistent with the interpreta¬tion that on the base branch of the hysteresis loop, a slow transition change is observed after the pH is raised by addition of portions of base. From the experimental, it can be observed that the metastability is predominantly due to the energy barriers preventing the equilibrium transition of the partially protonated milk protein system above a critical pH value (pHC). Another example of the metastability represented in this study is the slow disorder increasing of both casein components and whole caseins with heating tem-perature of milk. Then in a narrow range of temperature, a total breaking down of the arrangement (coagulation) can be observed. This state is called the conformational or phase transition. UHT heating of milk suppresses this transition process depending on both temperature and time of heating. How¬ever increasing of heat strength shifts the transition pH (pHC) to lower values. It seems certain that, the complex formation of casein / whey proteins at elevated temperatures plays a great role in heat stability of milk. It may be possible in future work to correlate the free energy parameters defined in this theory with casein composition and intermolecular

References

El-Shobery MAE. 1983. Effect of thermic procedures on the protein system of milk. Ph.D. thesis, Technical University of Munich, West Germany.

Kirchmeier O. 1977. Ergebnisse titrimetrischer Untersuchungen von Milch und Milchproducten. Milchwissenschaft, 32: 583.

Kirchmeier O. 1979. Titrimetric studies on milk and milk products. Milchwissenschaft 54: 156 [C.F. J. Dairy Res. 46: 397].

Kirchmeier O and El-Shobery MAE. 1981. Unpublished data. Technical University of Munich, West Germany.

Mansour AI. 2008. Relaxation time as indicator of buffering power of milk during Ultra-High-Temperature heating. Egypt. J. of Appl. Sci., 23 (6A): 185.

Neumann E. 1973. Moleculare Hysteresis und ihre kypernetische Bedeutung. Angew. Chem. 85: 430.

Neumann E and Katchalsky A. 1975. Thermynamic study of hysteresis in system of polyriboadenyl-polyribouridyl acid. Deutschen Bunsen-Gesellschaft for physical chemistry, 10: 185.

Pohl PM. 1969. Pebs Letters 5, 60. (North-Holland publishing company, Amesterdam) [C.F. Pohl, P. M. (1972). Angew. Chem. 84: 931].

Pohl PM. 1972. Kooperative Konformationsänderungen von globulären Proteinen. Angew. Chem. 84: 931.

Revzin A, Neumann E, Katchalsky A. 1975. Metastable secondary structures in ribosomal RNA. Molecular hysteresis in the acid-base titration of Escherichia coliribosomal RNA. J. Mol. Biol. 79: 95.

Rowland SJ. 1958. Determination of the Nitrogen Distribution in Milk. Jour. Dairy Res., 9: 42.

Schwarz G and Engel J. 1972. Kinetik Kooperativer Konformationsumwandlungen von linearen Biopolymeren. Angew. Chem., 84: 615.

Spodheim M, Neuman E. 1975. Ionic strength dependence of the hysteresis in the polyriboadenylate-polyribouridylate system. Biophys. Chem. 3: 109.