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Casein micelle dispersions into water, NaCl and $CaCl_2$: physicochemical characteristics of micelles and rennet coagulation

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Abstract

We have studied the effect of ionic strength from about 0 to 0.12 M on physicochemical characteristics of micelles and on gelation by rennet. Native phosphocaseinate powder was used as it exhibits attractive technological properties. The powder was dissolved at 25 g l^{-1} casein concentration with 0.2 g l^{-1} sodium azide into deionised water, NaCl or $CaCl_2$ solutions, at ionic strength from 0 to 0.12 M. The pH of micelle suspensions was corrected to pH 6.6 and, after overnight equilibration at 20°C, pH values were checked and eventually corrected. After re-equilibration, calcium and phosphorus partition, micelle size, centrifuged pellet hydration were determined and κ-casein hydrolysis and gelation of the suspensions were followed after rennet addition. The increase in NaCl concentration of casein micelle suspensions resulted in calcium and phosphorus solubilisation from micelles, and increase in pellet hydration. In the same way, the first-order rate constant of the κ-casein hydrolysis with rennet was reduced, while CMP at the clotting time determined by Formagraph was unchanged. Rennet gelation was consequently retarded by NaCl increase. Suspensions of casein micelles into $CaCl_2$ solutions did not exhibit calcium binding onto the micelles, as in milk, but a small reduction of diffusible phosphorus from 1.55 to 0.24 mM was shown. Pellet hydrations were unchanged. The increase in ionic strength with $CaCl_2$ retarded κ-casein hydrolysis, with an enhanced effect compared to NaCl study. As the CMP at the rennet clotting time was strongly reduced by $CaCl_2$ addition, the effect of $CaCl_2$ increase is typically biphasic, with an initial decrease and a following increase in rennet clotting times. From a technological point of view, reduction of ionic strength of milk led to reduced rennet clotting time. Results are discussed in terms of physicochemical modifications of milk micelles and the effect of ionic strength on enzymic reaction.

Keywords: Ionic strength; NaCl; $CaCl_2$; Rennet coagulation

1. Introduction

Many studies have investigated the effect of ionic strength increase of milk, through NaCl or $CaCl_2$ addition, on physicochemical characteristics of milk and on rennet coagulation (Dalgleish, 1983; Ramet, El-Mayda & Weber, 1983; McMahon, Brown, Richardson & Ernstrom, 1984; Grufferty & Fox, 1985; Van Hooydonk, Hagedoorn & Boerrigter, 1986; Zoon, Van Vliet & Walstra, 1989; Le Graet & Brulé, 1993), but few deal with the effect of the reduction of ionic strength towards zero.

In the current work, we intended to study the effect of ionic strength reduction from 0.12 to about 0 M with NaCl or $CaCl_2$ and an enriched-micelle caseinate called native phosphocaseinate powder (NPCP) on rennet coagulation of milk. Dispersion of this powder into water leads to an ionic strength close to zero and to shorter rennet clotting times, while dispersion into milk ultrafiltrate (UF) leads to properties very close to milk (Famelart, Lepesant, Gaucheron, Le Graet & Schuck, 1996).

2. Material and methods

2.1. Micelle dispersions

The same NPCP as that used by Famelart et al. (1996) was dispersed at 50°C using of a Sorvall agitator at a final casein concentration of 25 g l^{-1} into NaCl or $CaCl_2$ solutions prepared in deionised water, at an ionic strength from ~0 to 0.12 M. Rennet coagulation was

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studied with NPCP dispersed into milk ultrafiltrate (UF) as a control sample. NPCP was not soluble in $CaCl_2$ solutions. Hence, when the effect of ionic strength was studied through $CaCl_2$ addition, the dispersions were reconstituted into water at a final volume of 400 ml instead of 500 ml. Fifty ml of $CaCl_2$ solution at pH 6.6 was slowly added to reach final concentrations from 0 to 0.04 M (ionic strength from 0 to 0.12 M) and the pH was simultaneously reduced from about 7.4 to 6.6 with 0.5 M HCl. The pH value was adjusted to pH 6.6 at 30°C with 1 M HCl after 1 h equilibration and the final volume was adjusted to 500 ml. Sodium azide (0.2 g l^{-1} final) was added.

Dispersions were equilibrated overnight at 30°C, and used after an eventual pH correction and a 1 h re-equilibration at 30°C.

2.2. Physico-chemical analysis of dispersions

2.2.1. Mineral partition

Calcium and phosphorus concentrations were determined as in Famelart et al. (1996) in dispersions (total minerals) and in Centriflo CF 25 (MMCO 25 000 Da) ultrafiltrates (soluble minerals). Diffusible concentrations of minerals were obtained as in Famelart et al. (1996). Micellar concentrations were obtained by subtracting the diffusible from the total mineral concentrations.

2.2.2. Pellet hydration

Ultracentrifugation was performed at 30°C for 2 h at 75 000 *g*. Supernatant was carefully removed with syringe, and the drained pellet was dried at 103°C for 7 h. Solvation was determined as g of water per g of dry pellet.

2.2.3. Casein solubilisation

Optical density (OD) measurement at 280 nm of the 75 000 *g*-supernatant was performed after adequate dilution with 10 mmol l^{-1} EDTA solution at pH 10. OD was corrected for dilution and calculated in percent of OD of the dispersion. This was called the percent of solubilised casein.

2.3. Rennet coagulation kinetics

Rennet solution was prepared as in Famelart (1994). The resulting solution was stored frozen and used at 1 ml·l^{-1} of dispersion, immediately after thawing. For the renneting of small volumes of dispersions (10 ml), a 1/10 dilution of rennet solution with water was required, and 100 µl of this rennet dilution was used immediately for 10 ml of dispersion. Gelation measurements at 30°C were performed using a Formagraph (Foss Electric, Denmark), with at least 3 repetitions at the same trial for each dispersion to obtain a value for the clotting time, *r*. A Carri-med rheometer was used in oscillation mode, as in Famelart (1994), with measurements of shear moduli beginning at the clotting time determined using the Formagraph. Data were fitted with the Scott-Blair and Burnett model (Scott-Blair & Burnett, 1963) and allowed the determination of G_∞, the final modulus, k_{scot}, the time constant at which $G \propto /e$ was reached and t_{scot}, the time at which *G* began to increase, either for G' or G''.

2.3.1. Measurements of rennet hydrolysis

Caseinomacropeptide (CMP) concentration of renneted dispersions was determined by a chromatographic method at appropriate time intervals, at 30°C, as in Famelart (1994). The napierian logarithm of κ-casein was linearly related to the reaction time, and the absolute value of the slopes allowed the determination of the enzymic first-order rate constant, k_{enz}, in s^{-1}. Each dispersion was renneted in duplicate.

3. Results

3.1. Effect of NaCl increase on dispersion characteristics

Reconstitution of micelles into aqueous phases of increasing NaCl concentrations led to an increase in calcium and phosphorus concentrations of the ultrafiltrates (Fig. 1). The micellar calcium concentration was related to the micellar inorganic phosphorus by the following relation expressed in mM:

$$\text{Micellar calcium} = 3.0312 \times \text{Micellar inorganic phosphorus} - 7.9706.$$

Increasing NaCl concentrations from ~0 to 0.12 M led to an increase in micellar pellet hydration from 1.5 to 1.9 g of water per g of dry pellet (Fig. 2) and to an increase in casein concentrations of ultracentrifuged supernatants from 4.5 to 7.5% of total OD of non-centrifuged dispersions (Fig. 2).

3.2. Effect of NaCl increase on rennet coagulation

The enzymic first-order rate constant, k_{enz}, decreased approximately linearly with the addition of NaCl to the aqueous phase from 2.5×10^{-3} to 1.5×10^{-3} s^{-1} (Fig. 2). The rennet clotting time observed by Formagraph increased linearly from about 500 to 1100 s with NaCl increase (Fig. 2). The proportion of total CMP released at the formagraph-clotting time was around 78%, and did not show any variation with NaCl concentrations. The time constant, t_{scott} deduced from the Scott-Blair and Burnett model increased linearly with NaCl concentrations from 300 to 800 s, while the rate constant, k_{scott}, increased from about 1000 to 2000 s (results not shown). G_∞ did not show any variation with NaCl concentrations (results not shown).

k_{enz} and clotting time for dispersion into UF were close to NaCl values, compared at the same ionic strength

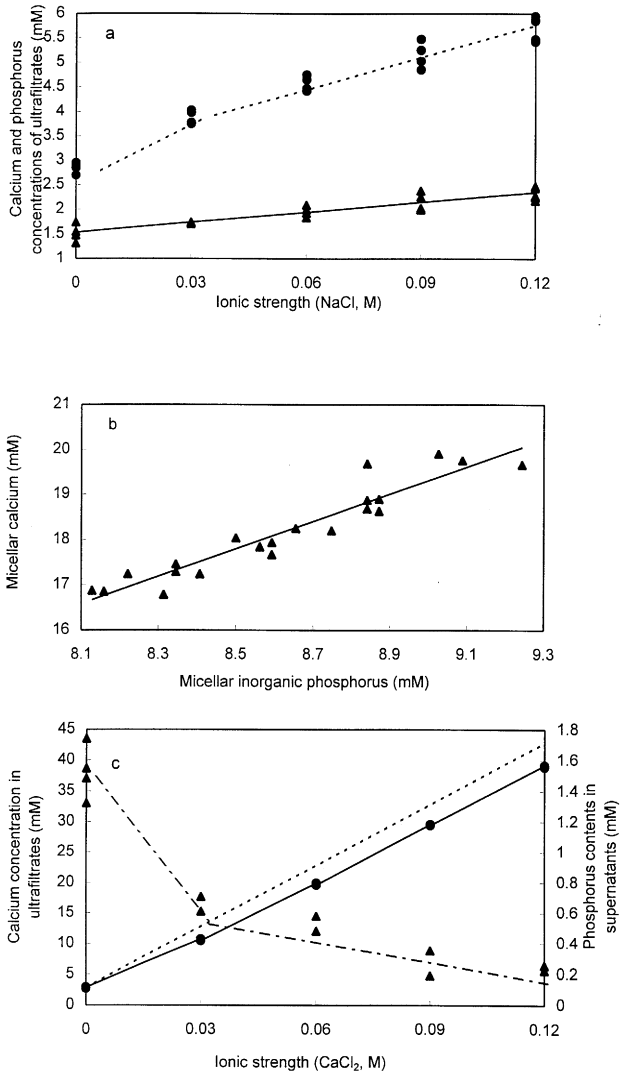

Fig. 1. (a) Calcium and phosphorus concentrations in ultrafiltrate of a casein micelle dispersion into NaCl solutions from ~0 to 0.12 M. (- - -●- - -), calcium; (—▲—), phosphorus. (b) Micellar calcium versus micellar inorganic phosphorus in casein micelle dispersions into NaCl solutions. From left to right, the NaCl concentration decreased from 0.12 to ~0 M. (c) Calcium and phosphorus concentrations in ultrafiltrate of casein micelle dispersion into $CaCl_2$ solutions of ionic strength from ~0 to 0.12 M. (—●—), calcium; (– - –▲– - –), phosphorus. The dotted line (- - -) represents the calculated calcium concentration assuming that all added calcium stayed in the diffusible phase.

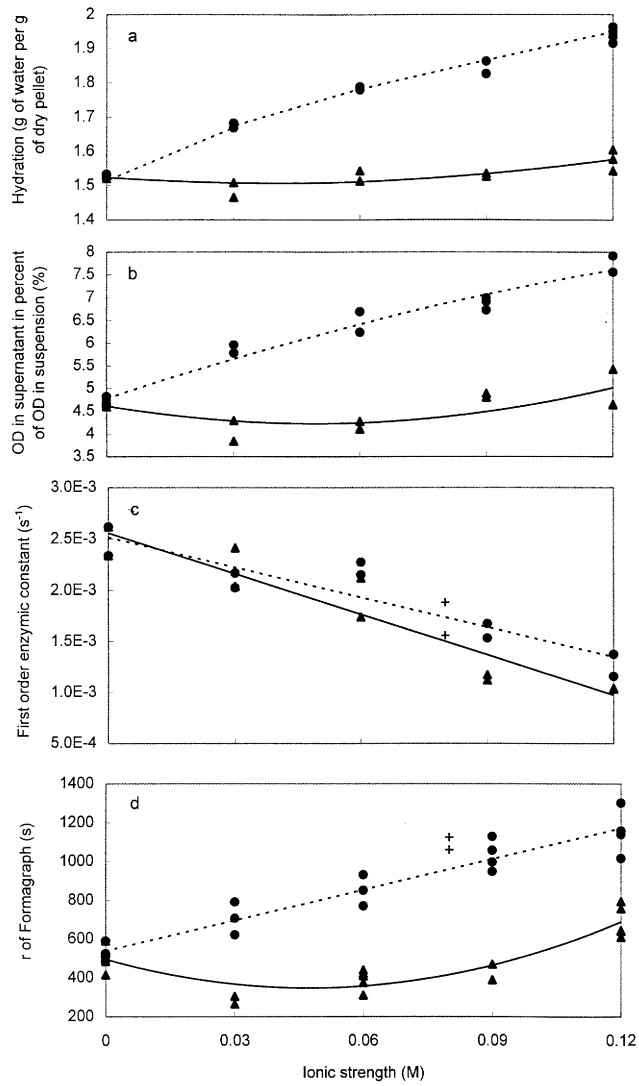

Fig. 2. Physicochemical changes obtained on dispersion of casein micelles in salt solutions of increased ionic strength. (- - -●- - -), NaCl; (—▲—), $CaCl_2$; (+), milk ultrafiltrate (a) Pellet hydration versus ionic strength. (b) Optical density (OD) in supernatant in percent of total OD. (c) First-order enzymic constant. (d) *r*, the clotting time from formagraph.

(Fig. 2). The percent of total CMP released at the formagraph-clotting time for dispersion into UF was very close to the value for dispersion into NaCl (83%), within the experimental error.

3.3. *Effect of $CaCl_2$ increase on dispersion characteristics*

Addition of $CaCl_2$ led to a decrease in phosphorus concentrations of ultrafiltrates from 1.5 to 0.2 mM (Fig. 1). This decrease was greater over the range of $CaCl_2$ additions between ~0 and 0.010 M. The increase in $CaCl_2$ concentrations led to an increase in calcium concentrations of ultrafiltrates (Fig. 1). Almost all of the added calcium stayed in the aqueous phase. In Fig. 1 the theoretical curve of calcium concentrations in the ultrafiltrate as a function of ionic strength has been constructed assuming no exchange between added calcium and the colloidal phase. The discrepancy between this model and the experimental data showed reduced soluble calcium values, mainly at 0.01 M $CaCl_2$ addition. This soluble calcium reduction varied from 2.3 mM (0.01 M $CaCl_2$) to 3.8 mM (0.04 M $CaCl_2$), while the reduction in soluble phosphorus was from 0.86 to 1.3 mM, respectively. The ratio of transferred calcium/phosphorus varied from 2.6 to 3. Addition of $CaCl_2$ led to only small

changes in pellet hydration and casein solubilisation: a slight decrease at about 0.02 M of added calcium, followed by a slight increase (Fig. 2).

3.4. Effect of $CaCl_2$ increase on rennet coagulation

Addition of $CaCl_2$ led to a linear decrease in the first-order enzymic constant, k_{enz}, similar to the case of NaCl addition, from about 2.5×10^{-3} to 1.2×10^{-3} s^{-1} (Fig. 2). The rennet clotting time decreased from 500 to 300 s, at 0.020 M $CaCl_2$, and then increased towards 700 s at 0.04 M $CaCl_2$ (Fig. 2). The proportion of total CMP released at the clotting time decreased strongly from 78% to 45% with the addition of 0.010 M $CaCl_2$, and stayed at 45% at higher $CaCl_2$ concentrations. The time constant, t_{scott} deduced from the Scott-Blair and Burnett model was constant, at 300 s, from 0 to 0.02 M $CaCl_2$ and then increased to 400 s at 0.04 M $CaCl_2$. The rate constant, k_{scott}, first decreased from 900 s without $CaCl_2$ to 700 s at 0.01 M $CaCl_2$ and then increased to 2000 s (results not shown). G_∞ did not show significant variation with $CaCl_2$ addition (results not shown).

4. Discussion

Dispersing micelles into NaCl solution led to a solubilisation of calcium and phosphorus, while NaCl addition into milk leads only to a calcium solubilisation (Grufferty & Fox, 1985; Van Hooydonk et al., 1986; Zoon et al., 1989; Le Graet & Brulé, 1993). Phosphorus solubilisation was confirmed by the linear relation observed between micellar calcium and micellar inorganic phosphorus. Part of the solubilised calcium might be bound to phosphorus and the remainder might be directly bound to caseins. In milk, an increase in ionic strength leads also to the increase of the stoichiometric solubility product of calcium citrate salts and their dissociation into citrate and calcium ions. The calcium ions will restrain the dissociation of calcium phosphate, so that the increase in diffusible phosphate is undetectable. This suggestion could explain the difference between milk and the present dispersions. NaCl addition not only increased ionic strength, but also led to exchange of sodium with colloidal calcium and to great changes in the micellar structure (Fig. 2). Calcium solubilisation in the present dispersions was 4-fold that observed in Grufferty and Fox (1985).

By contrast, $CaCl_2$ addition did not lead to major modifications in the micellar structure, and almost all the added calcium remained in the soluble phase, with ionic strength increase. The high values for the ratio of transferred calcium/phosphorus was far from the 1.5 ratio found in milk after a 3 mM $CaCl_2$ addition (Van Hooydonk et al., 1986), but the soluble phosphorus concentrations found in the present study were 1/10 of that in milk. The limiting factor was probably the diffusible phosphorus content, as it was close to zero at 0.12 M ionic strength (Fig. 1). Calcium addition led to a binding with casein, while, in milk, it displaces soluble calcium phosphate salts into insoluble calcium phosphate.

Casein solubilisation and hydration showed slight biphasic changes with $CaCl_2$ addition. Between $\sim$0 and 0.01 M $CaCl_2$, binding of calcium occurred, leading to a reduction in the negative charge of casein. Hydration was slightly reduced and some individual caseins possibly return to the colloidal phase, due to repulsive force reduction between protein chains. Beyond 0.01 M $CaCl_2$, transferred calcium increased more slightly, but the main effects related to the ionic strength increase. The reduction of the stoichiometric pK_a with ionic strength led to the increase of the negative charge on casein, with a hydration increase and casein dissociation due to the repulsion increase.

Addition of salts to micelle dispersions led to a decrease of enzymic rate, while addition of $CaCl_2$ led to a reduction of the CMP released at the clotting time. A reduction of CMP at clotting time in the presence of added $CaCl_2$ in milk has been observed previously (Bringe & Kinsella, 1986; Van Hooydonk et al., 1986). Hence, the clotting time showed a minimum around 0.02 M $CaCl_2$, while it increased linearly between 0 and 0.12 M NaCl. Our results on enzymic rate and clotting time with NaCl addition agreed with the literature (Ramet et al., 1983; Van Hooydonk et al., 1986; Zoon et al., 1989; Grufferty & Fox, 1985), though a minimum in clotting time around 0.050 M NaCl has been reported by Alais and Lagrange (1972). According to Dalgleish (1992), ionic strength increase would lead to the screening of negative charge on both enzyme and substrate and to the increase in the rate of proteolysis of κ-casein, while according to Visser, Van Rooijen and Slangen (1980), ionic strength increase would lead to a screening of the positively charged cluster of κ-casein and of chymosin, which would bring about a decreased enzymic rate. The increase in $CaCl_2$ concentration of milk is known to, either increase enzymic rate at 0.008 M $CaCl_2$ addition (Bringe & Kinsella, 1986), or to be without effect (Scott-Blair & Oosthuizen, 1961; Van Hooydonk et al., 1986). Addition of $CaCl_2$ in milk leads to either a decrease in clotting time (Dalgleish, 1992), or a minimum at 0.040 M $CaCl_2$ (McMahon et al., 1984), while an increase in aggregation rate at 0.002–0.008 M $CaCl_2$ is cited by Dalgleish (1983) and Bringe and Kinsella (1986). The reduction of CMP at clotting time, which is indicative of an increase in the aggregation rate, was probably related to the binding of calcium and phosphorus to micellar casein, and to a reduction of its negative charge.

Changes in clotting time were related to casein hydration and solubility changes, while enzymic hydrolysis rates were not related to changes in casein hydration. Enzymic rate did not seem to be influenced by the state of

compaction or of protrusion of casein chains, whereas the aggregation rate depended on it.

From observations made in this study, native phosphocaseinate constitutes an attractive material to evaluate the effect of environmental conditions on milk micelles.

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