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Studies on Camel Casein Micelles Treatment with Soluble and Immobilized Pepsin

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ABSTRACT. Isolated camel casein micelles were treated with pepsin, in both soluble and immobilized forms, to study the location and distribution of glycosylated portion (glyco-κ-casein-like component) of camel casein micelle. The release of nonprotein nitrogen and sialic acid soluble in 2% and 12% trichloroacetic acid (TCA) was studied. Soluble pepsin released a maximum amount of 5.62 and 4.97 mg NPN/g casein soluble in 2% and in 12% TCA, and 7.1 mg sialic acid/g casein soluble in 2% or 12% TCA, respectively. The corresponding figures for immobilized pepsin were 4.97, 4.30 and 6.7. It was concluded that almost (90%) of glycosylated portion of camel casein micelle is on the micelle surface.

Casein in the milk of most mamalia including that of camels occurs as a spherical biocolloid, called micelle, which range in diameter from approximately 14 to greater than 600 nm (Ali and Robinson 1985 and Holt 1985). The spatial relationships of individual casein components within a micelle are of fundamental importance in understanding micelle stability and bioassembly. The nature of the bovine casein micelle and the factors governing its stability have been investigated intensively for many years (Mehaia 1983, Schmidt 1980, Slattery 1976 and McMahon and Brown 1984), and several conflicting models have been proposed (Mehaia and Cheryan 1983a, 1983b, Schmidt 1980, McGann *et al.* 1980, Slattery 1978, Parry and Carroll 1969, Cheryan *et al.* 1975 and Kudo *et al.* 1979). It is now known that there are two forms of κ-casein, one form containing varying amounts of carbohydrate, known as "glycosylated κ-casein" and another form devoid of carobhydrate, known as "non-glycosylated κ-casein" (Mehaia and Cheryan 1983a, 1983b, Slattery 1978 and Dalgleish, 1985).

No information is available in the literature on the structure of camel casein micelle. However, there are four reports on separation of major components of camel milk casein (Farah and Farah-Riesen 1985, El-Agamy 1983, Ottogalli and Resmini 1976 and Dedek *et al.* 1978) and one report on the size distribution of camel casein micelle (Ali and Robinson 1985). Although κ-casein has never been isolated in a pure state from camel milk, but the addition of rennet to camel milk caused a clotting reaction with a coagulum (Woodward 1976 and Farah and Farah-Riesen 1985). This suggests the possible occurrence of κ-casein or a homologous protein in camel milk.

In view of studying the structure of camel casein micelle, it was considered important to determine whether or not glycosylated portion of camel casein micelle (glyco-κ-casein-like component) is located on the surface of the micelle. Mehaia and Cheryan (1983a, 1983b) have described a new approach, using soluble and immobilized enzymes, to study the location and distribution of glycosylated κ-casein of bovine casein micelle. Soluble pepsin is expected to penetrate the micelle and react with all of glycosylated portion of the micelle (Fig. 1A), whereas, immobilized pepsin being much larger than the casein micelle, will not penetrate it but instead reacts only with the micelle surface (Fig. 1B). As part of our continuing

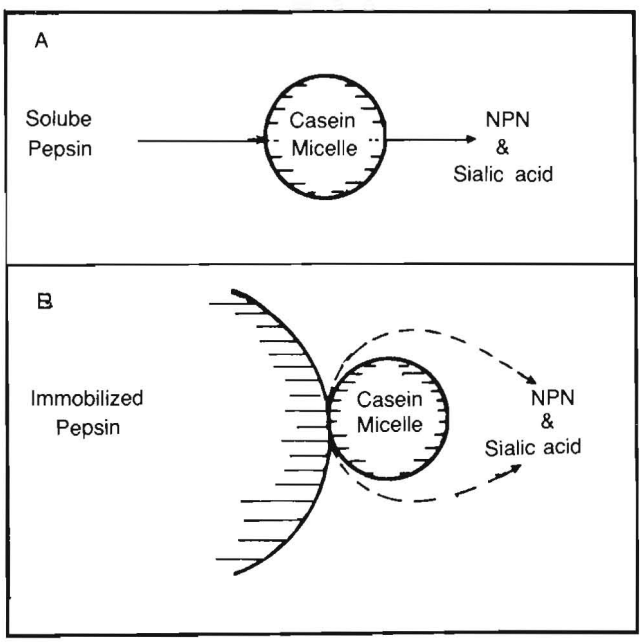

Fig. 1. Schematic representation of nonprotein nitrogen and sialic acid by the action of (A) soluble pepsin, (B) immobilized pepsin.

studies on camel casein micelle, in the present study, the same approach has been applied to locate glycosylated portion of camel casein micelle using soluble and immobilized pepsin.

Materials and Methods

Enzymes

Pepsin (EC 3.4.23.1) was purchased from Sigma Chemical Company, St. Louis, MO, USA. Immobilized pepsin was prepared by procedure of Mehaia and Cheryan (1983a).

Isolation of Casein Micelles

During the isolation of micelles, care was taken to prevent the milk or micelles from cooling below 20°C to prevent dissociation of the micelle. According, raw milk was obtained from individual Najdi camels (*Camelus dromedarius*) in the private farm near Riyadh City, Saudi Arabia, and skimmed immediately by centrifugation at 4000 X g for 30 min. The skim milk then was centrifuged at 6000 X g for 60 min in Beckman L8-80 ultracentrifuge. The supernatant was discarded, and the protein pellet was twice washed by suspending it in the UF-permeate of the same skim milk and recentrifuging. After the last wash the pellet was re-suspended in UF-permeate to a normal casein level of about 2 g/100 ml. Ultrafiltration of skim milk was carried out at 30°C by using a hollow fiber from Amicon (Danvers, MA, USA) and H1P1-43 membrane. To minimize microbial growth, thimerosal (Sigma Chemical Co.) was used at a concentration of 100 ppm in the reaction mixture (Mehaia and Cheryan 1983a).

Treatment of Casein Micelles with Enzymes

The casein micelle suspension was equilibrated to 37°C, pH 6.6. The appropriate amount of soluble or immobilized pepsin that would result in a clotting time of 25 to 30 min was added. The reaction vessel was placed in a reciprocating shaker water bath. At various intervals, 10 ml samples of reaction mixture were removed and 40 ml of trichloroacetic acid (TCA) added to give 2% or 12% concentration. They, then, were filtered through whatman No. 42 filter paper and the filtrates were analyzed for nitrogen and sialic acid. Controls were prepared to test for any microbial activity, for any solubilization of the immobilized pepsin and for any solubilization of micelles over the reaction period, as described by Mehaia and Cheryan (1983a).

Analytical Methods

Protein and nitrogen (N) were determined using Micro-Kjeldahl. Unless otherwise mentioned, protein contents is N × 6.38. Resorcinol (Svennerholm

1957) and thiobarbituric acid (Warren 1959) methods are used to measure sialic acid. A standard curve was prepared with n-acetyl-neurminic acid (Sigma Chemical Co.).

Results and Discussion

Studies similar to those described, in previous report (Mehaia 1987), with chymosin were also conducted using pepsin as the enzyme.

Macropeptides Released by Pepesin

The release of non-protein nitrogen (NPN) and peptides from camel casein micelles during the action of soluble and immobilized pepsin is depicted in Figs. 2 and 3. With both soluble and immobilized pepsin, the initial rates of release, as

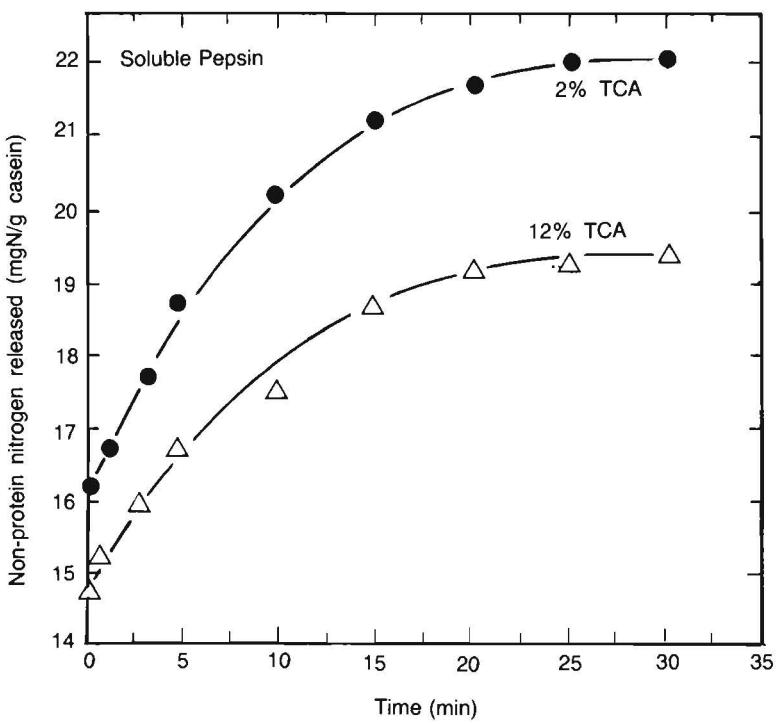

Fig. 2. Release of nonprotein nitrogen from camel casein micelles by soluble pepsin at pH 6.6 and 37°C. Data are means of three replicate experiments.

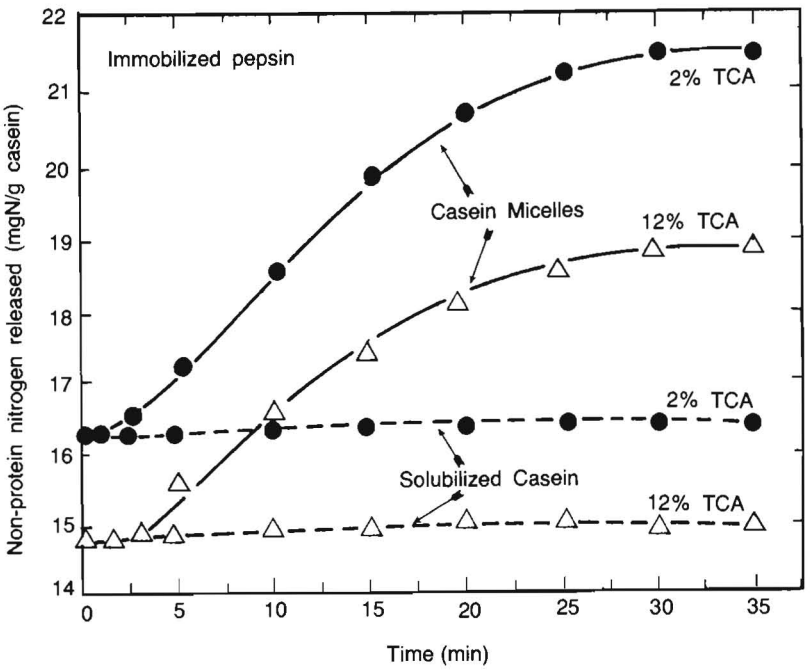

Fig. 3. Release of nonprotein nitrogen from camel casein micelles and from casein solubilized from micelles during the reaction period by immobilized pepsin at pH 6.6 and 37°C. Data are means of three replicate experiments.

well as the maximum amount released (total NPN in reaction mixture minus NPN at zero-time and NPN from control experiments), of the glycomacropeptide (NPN in 2% TCA) is much greater than that of the non-glycomacropeptide (as measured by the difference between NPN in 2% and NPN in 12% TCA), see Table 1. The concentration of NPN-12 compared to the total NPN (NPN-2) were 82% and 86%

Table 1. Macropeptides released from camel casein micelles using soluble and immobilized proteases, (mg NPN/g casein)[a].

Macropeptide	*S.chymosin*[b]	*I.Chymosin*[b]	*S.Pepsin*	*I.Pepsin*
A. Glyco+nonglyco (NPN-2)	5.11	4.62	5.62	4.97
B. Glyco (NPN-12)	4.31	4.11	4.64	4.30
C. Nonglyco (A-B)	0.80	0.51	0.98	0.67

a Data are means of three replicate experiments.
b From Mehaia (1987)

using soluble and immobilized pepsin, respectively. However, the maximum NPN-12 released by immobilized pepsin was about 92% of that released by soluble pepsin. Since immobilized pepsin can not penetrate the casein micelles, the results suggest that both glyco- and non-glyco-κ-casein-like component are available to immobilized pepsin and presumably are on the micelle surface, with at least 90% of the micelle's glycosylated portion on its surface. These results agree with previous report using chymosin (Mehaia 1987) and with other studies on the bovine casein micelles (Mehaia and Cheryan 1983a, 1983b, Kudo *et al.* 1979 and Slattery 1978).

Sialic Acid Released by Pepsin

Sialic acid is frequently used as a measure of the carbohydrate content of κ-casein or of the macropeptide released by proteases. Figures 4 and 5 show the sialic acid released by the action of soluble and immobilized pepsin. Both figures show the rate of, and the maximum increase in, sialic acid in the 2% TCA filtrate was almost the same as that obtained in the 12% TCA filtrate (Mehaia 1987 and Mehaia and Cheryan 1983a, 1983b). Maximum amount of sialic acid released in

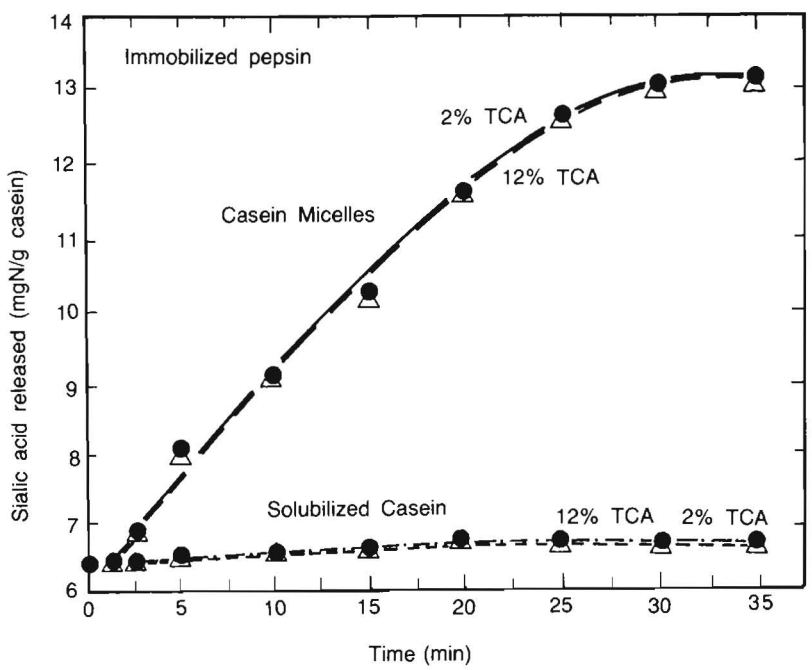

Fig. 4. Release of sialic acid from camel casein micelles by soluble pepsin (under the same conditions as Fig. 2).

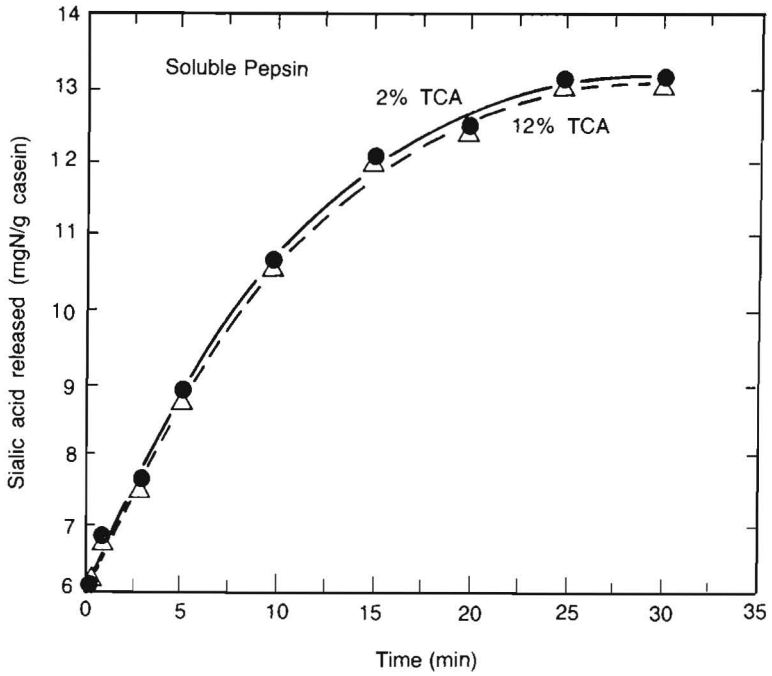

Fig. 5. Release of sialic acid from camel casein micelles and from casein solugilized from micelles during the reaction period by immobilized pepsin (under the same conditions as Fig. 3).

these experiments are shown in Table 2. The maximum sialic acid released by immobilized pepsin was about 94% of that released by soluble pepsin. This appears to confirm the above data regarding the maximum NPN released.

Table 2. Sialic acid released from camel casein micelles using soluble and immobilized proteases[a,b]

Enzymes	Sialic acid (mg/g casein)
Soluble Chymosin	7.2[c]
Immobilized chymosin	6.8[c]
Soluble pepsin	7.1
Immobilized Pepsin	6.7

a Data are means of three repliate experiments.
b Total sialic acid in isolated casein micelles = 7.35 mg/g casein.
c From Mehaia (1987).

Tests for microbial activity and for "soluble" activity of immobilized pepsin indicated that there was no increase of non-protein nitrogen and/or sialic acid in the TCA filtrates, *i.e.* there was no significant microbial activity over the reaction period and there was no free "soluble" pepsin activity in the immobilized pepsin. However, there was a slight increase in NPN and/or sialic acid during the time of the experiments (Figs. 3 and 5), this is due to casein solubilized control. Thus, a net release of NPN and/or sialic acid by the action of immobilized pepsin was obtained by subtracting the solubilized casein NPN and/or sialic acid from the total NPN and/or sialic acid released; the data in Tables 1 and 2 accounts for the solubilization.

Chymosin vs. Pepsin-Micelle Interactions

Results obtained with pepsin-micelle interactions were similar to those with chymosin (Tables 1 and 2), however, pepsin released more NPN than chymosin. This may be due to the fact that pepsin is not as specific as chymosin, and as a result, peptides other than macropeptide are released from κ-casein-like component. Sialic acid released by soluble proteases was about 96% of that in the isolated camel casein micelles, this has also been previously reported by others (Kim *et al.* 1967, Waugh and Talbot 1971 and Mehaia and Cheryan 1983b) using bovine casein micelles.

In conclusion, the obtained results appear to indicate that both forms of κ-casein-like component of camel casein micelle, exist on the micelle surface, and the surface is highly glycosylated. Further analysis of the camel casein fractions, and of their position in the micelle network, is needed in order to explain the nture of the camel milk casein and their chemical and physical behaviour.

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دراسات على جسيمات كازين حليب الإبل المعالجة بأنزيم الببسين الذائب والمثبت (غير الذائب)

محمد عبدالفتاح مهيا

قسم علوم الأغذية ـ كلية الزاعة ـ جامعة الملك سعود ـ الرياض ـ المملكة العربية السعودية

تم في هذه الدراسة معالجة جسيمات كازين حليب الإبل ـ المعزولة من حليب فرز ـ بأنزيم الببسين بصورتيه الذائبة والمثبتة وذلك بغرض دراسة موقع وتوزيع الجزء المحتوى على كربوهيدرات (المكون المشابه للجليكو ـ كابا ـ كازين) في جسيمات كازين حليب الإبل. وقد تمت دراسة النيتروجين غير البروتيني وحامض السياليك الذائب في كل من ٢٪، ١٢٪ ثلاثي كلوروحامض الخليك، ووجد ان أقصى الكميات المنطلقة بواسطة أنزيم الببسين الذائب كانت كما يلي: ٥,٦٢، ٤,٩٧ ملليجرام نيتروجين غير بروتيني لكل جرام كازين، ذائب في ٢٪، ١٢٪ ـ ثلاثي كلوريد حامض الخليك، على التوالي. بينما كمية حامض السياليك المنطلقة والذائبة في ٢٪ أو ١٢٪ ثلاثي كلوريد حامض الخليك كانت ٧,١ ملليجرام لكل جرام كازين. ان الأرقام المتماثلة الناتجة من إستخدام أنزيم الببسين المثبت كانت كما يلي: ٤,٩٧، ٤,٣٠، ٦,٧.

يتضح من هذه الدراسة ان (٩٠٪) تقريباً من جزء جسيمات كازين حليب الإبل المحتوى على كربوهيدرات يوجد على السطح الخارجي للجسيمات بينما الجزء الباقي (حوالي ١٠٪) يكون موزعاً بداخل الجسيمات.