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INFLUENCE OF LYSOZYME ON THE ENZYMATIC COAGULATION OF MILK

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Key words: lysozyme, rennet coagulation of casein, electron microscopy, electrokinetic potential of casein micelles.

The influence of lysozyme on the rennet coagulation of casein, dispersed in various solutions, was investigated. The structure of coagulate precipitated from milk by lysozyme and the structure of lysozyme-rennet coagulate were determined by electron microscopy. The conducted experiments showed that lysozyme reduced the time of rennet coagulation proportionally to the amount added. Microscopic observations demonstrated a similarity of the structure of lysozyme coagulate and acid coagulate. The above results allow to draw the conclusion that the effect of lysozyme on casein is connected with a change in the electrokinetic potential of casein micelles in milk.

The process of rennet coagulation of milk protein and the influence of various compounds on the course of this reaction is the subject of many studies, which are, among other things, aimed at the development of new milk-coagulating enzymatic preparations [2, 9, 10].

The studies of Green et al. [3, 4, 5] indicate that the reduction of coagulation time by cations depends on the stability of their bonds with casein micelles and on the size of their positive charge. The cationic compounds which abbreviate effectively the time of rennet coagulation of casein include also lysozyme. This enzyme, which is present in almost all tissues and secretions of the human body, is considered as one of the major factors of non-specific resistance of organism [13]. Such function of lysozyme and its high content in human milk compared with cow's milk, is a back ground for studies on the utilization of lysozyme in the process of modification of cow's milk. These studies showed that lysozyme has also a certain influence on the casein of cow's milk, by making its degradation in infant's alimentary tract easier [12]. The mechanism of this influence is not fully explained. Probably the change of electrokinetic potential of casein micelle by lysozyme causes a change of its sensitivity to the action of digestive enzymes.

In connection with the above comments, the aim of the present work was to study the influence of lysozyme on the rennet coagulation of casein. A compari-

son was also made between the structure of a coagulate precipitated from milk by lysozyme, rennet and by acid. The results of the above observations may facilitate the interpretation of the effect of lysozyme on the casein of cow's milk.

MATERIAL AND METHODS

A. THE PREPARATION AND RESEARCH MATERIAL APPLIED

In the experiments, five-times crystallized lysozyme of hen egg white, produced by Sarva Feinbiochemica company and Hansen rennet with strength 1:25000, were used. The studies were conducted on skim-milk, casein of BDH company and casein micelles obtained by the ultracentrifugation of milk.

B. PREPARATION OF SAMPLES FOR ANALYSIS

As initially assumed the experimental part was implemented in two stages. In the first part of the work, the coagulation time of casein of lysozyme-modified cow milk, was determined. These experiments were performed on skimmed milk, solution of casein in milk dialyzate, artificial milk serum, in a suspension of casein and in an artificial solution of milk salts.

The solutions of salts were prepared according to the method of Jenness and Kops [7], recommended for studies on casein in model systems. Milk dialyzate was prepared by the dialysis of fresh skim cow milk against distilled water [3]. Dialysis was conducted at 4°C for 24 h. The content of calcium in dialyzate was supplemented to its level in milk, by the addition of calcium chloride. Casein micelles were obtained by centrifugation of fresh, skim-milk in a Beckman L-565 ultraseparator, with the application of rotor Ti 70-at acceleration 107×10^3 g during 1 hour at 1-2°C, concentration of lysozyme in the samples was 0.5; 1.0 and 2.0 mg/cm³, rennet was added in the form of 1% solution in an amount which ensured that the time of coagulation in the control samples (without lysozyme's addition) would be about 10 minutes. The time of coagulation was determined at 30 and 37°C. In this part of the work, the influence of preliminary incubation of samples with lysozyme on their coagulation time under the effect of rennet, was investigated. The preliminary incubation was conducted at 37°C for 10 and 20 minutes. In all experimental models, the time of rennet coagulation was determined by the visual method, according to Arentzen [1].

In the second part of the work, attempts were made to determine the structure of coagulate precipitated from milk by lysozyme (12 mg/cm³ milk) and of rennet coagulate of milk, treated with lysozyme (2 mg/cm³). For comparison, the structures of rennet and acid coagulate of milk were determined. The microscopic preparations were analysed under an electronic scanning microscope (SEM) JEOL-JSM S1 (working voltage 10 MeV) and electronic transmission microscope (TEM) Tesla BS-500.

RESULTS AND DISCUSSION

The comparison of coagulation time of lysozyme-modified casein dispersed in various solutions was aimed at determining the degree of reaction between casein and lysozyme, depending on the presence of other milk components. With a view to possible increase the sensitivity of lysozyme's effect. Various types of casein were used as substrate in the experiments, i.e. native casein micelles, soluble BDH casein and casein micelles isolated from milk by ultracentrifugation.

The performed measurements demonstrated that in all experimental model, lysozyme shortened the time of rennet coagulation of casein, proportionally to the amount of its addition (Tab. 1). It was also revealed that the differences between the time of rennet coagulation of the samples with lysozyme and the control samples were dependent both on the type of casein and the type of solution in which the micelles were dispersed. The smallest differences appeared in skim-milk: at 37°C the coagulation time of milk with an increasing addition of lysozyme was shortened in comparison with the control samples by 8.4; 17.8 and 38.8%. In an analogical experiment conducted on the solution of BDH casein in artificial milk serum, the discussed time was shorter by 30.6; 37.9 and 59.5% in comparison with the control sample. The greatest differences were obtained in case of casein micelles dispersed in a synthetic solution of milk salts. The coagulation time of these samples with the addition of lysozyme equalling 0.5; 1.0 and 2.0 mg/cm³ was shorter than that of the control sample by 23.8; 42.0 and 67.1% (Tab. 1). In further part on the experiment, no differences were practically stated in the sensitivity of casein samples to rennet, depending on the time of their preliminary incubation with lysozyme (Tab. 2).

Table 1. Changes in time of rennet coagulation of lysozyme-modified casein samples

Kind of sample	Time of coagulation (s)				Reduction of coagulation time in relation to the control sample %		
	control sample	samples with addition of lysozyme (mg/cm ³)			samples with addition of lysozyme (mg/cm ³)		
		0.5	1.0	2.0	0.5	1.0	2.0
A	538	493	442	329	8.4	17.8	38.8
B	541	487	411	318	10.0	24.2	41.2
C	569	511	408	301	10.2	28.4	47.2
D	384	267	238	155	30.6	37.9	59.5
E	404	307	234	133	23.8	42.0	67.0

- A skim-milk
- B casein (BDH) in milk dialyzate
- C casein micelles in milk dialyzate
- D casein (BDH) in artificial serum of milk
- E casein micelles in artificial serum of milk

Table 2. Influence of preliminary incubation of casein samples with lysozyme on the time of rennet coagulation

Kind of sample	Time of preliminary incubation (min)	Time of rennet coagulation (S)		
		addition of lysozyme (mg/cm ³)		
		0.5	1.0	2.0
A	0	653.4	602.3	408.5
	10	653.4	601.2	408.5
	20	653.0	600.3	418.5
B	0	620.2	561.0	483.0
	10	621.4	561.0	482.5
	20	621.5	561.0	482.4
C	0	640.2	567.0	468.0
	10	639.7	566.7	467.0
	20	639.7	567.2	467.7
D	0	388.2	308.7	207.5
	10	387.5	308.7	208.5
	20	387.0	308.0	208.5
E	0	425.8	320.8	209.2
	10	427.0	319.4	210.2
	20	427.0	320.8	211.2

A - skim-milk

B - casein (BDH) in milk dialyzate

C - casein micelles in milk dialyzate

D - casein (BDH) in artificial serum of milk

E - casein micelles in artificial serum of milk

The observations of Mulin and Wolf [11] and of Green [3] indicate also that lysozyme shortens the time of rennet coagulation of milk. Besides, high concentrations of this enzyme may cause a precipitation of casein from the solution.

In our studies of determination of firmness of rennet curd of milk treated with lysozyme it was observed that with the increase of lysozyme's addition till the moment of coagulate formation, the viscosity of milk was changing which may be a reflection of certain differences in the structure of the rising curd [12]. These observations inclined us to determine further the structure of coagulate of proteins of lysozyme-precipitated milk and also of lysozyme-rennet coagulate. The enclosed photographs (1, 2, 3, 4) show a microscopic picture (TEM) of rennet, acid, rennet-lysozyme and lysozyme coagulate. An analysis of the electronograms reveals that rennet coagulate represents a net of lined up molecules, with visible chains of single molecules and chains resulting from the not yet connected agglomerates. The degree of agglomeration of casein in case of acid coagulate was higher. Similarly, a higher agglomeration of molecules and chains may be observed in the microscopic picture of lysozyme-rennet coagulate in comparison with the rennet coagulate. It may be also noticed that the structure of lysozyme coagulate was similar to the picture of acid curd though the observed chains of

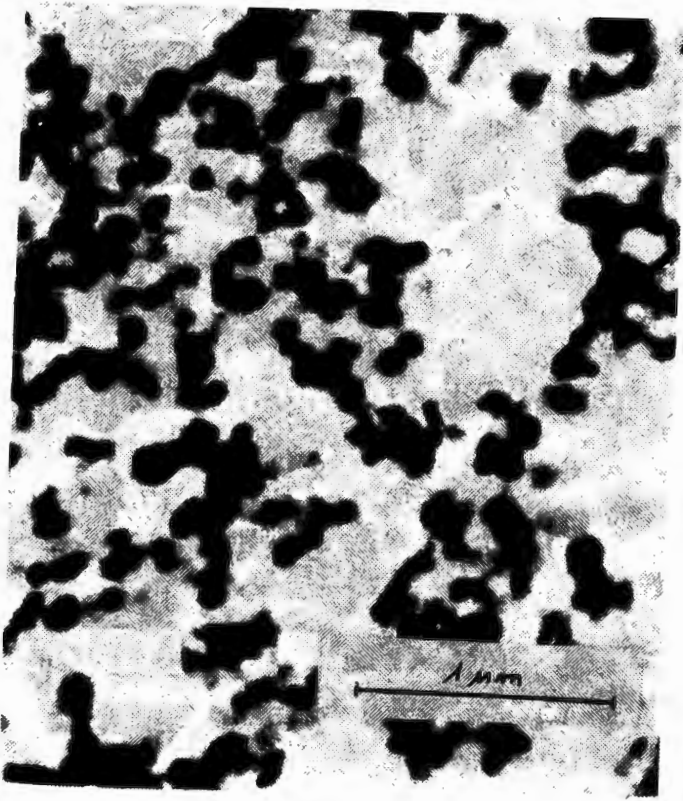


Fig. 1. Electronogram of rennet coagulate of milk (TEM)



Fig. 2. Electronogram of acid coagulate of milk (TEM)

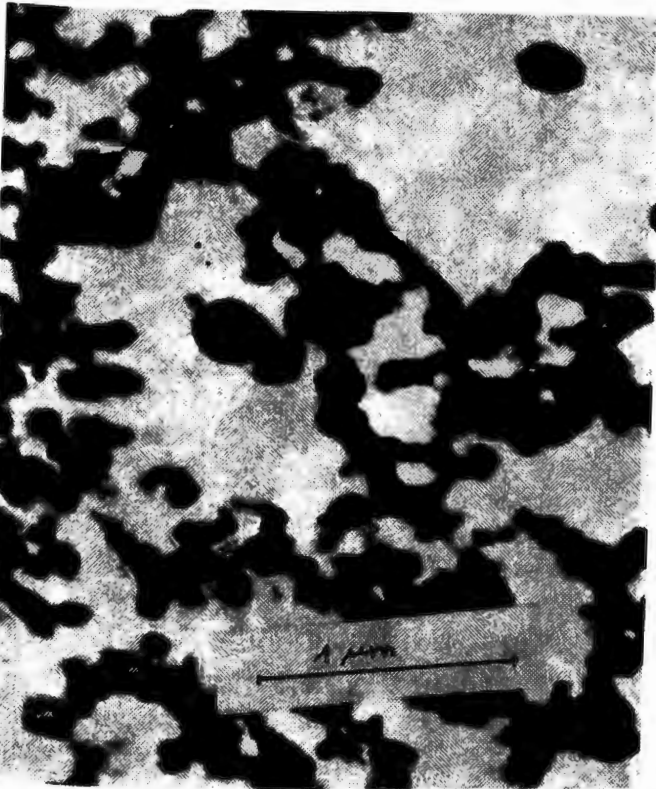


Fig. 3. Electronogram of rennet coagulate from milk treated with lysozyme (TEM)

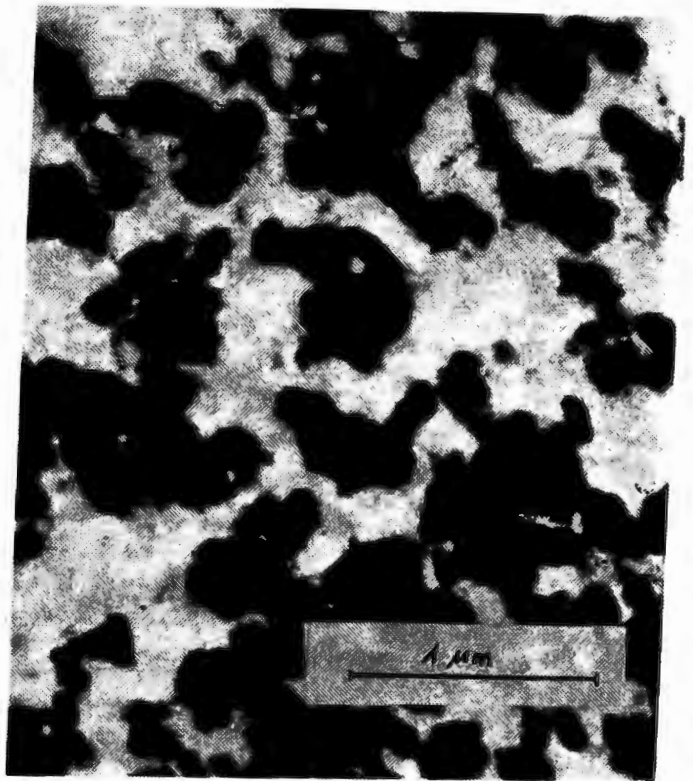


Fig. 4. Electronogram of coagulate precipitated from milk by the lysozyme (TEM)

casein seemed to be somewhat smaller. The greater agglomeration of casein in acid coagulate is probably connected with the rapid and almost complete neutralization of surficial charges of micelles [8]. Hence there is more complete agglomeration of molecules of rennet coagulate of milk, treated previously with lysozyme.



Fig. 5. Electronogram of rennet coagulate of milk (SEM)



Fig. 6. Electronogram of acid coagulate of milk (SEM)



Fig. 7. Electronogram of rennet coagulate from milk treated with lysozyme (SEM)



Fig. 8. Electrogram of coagulate precipitated from milk by the lysozyme (SEM)

It is possible that the similar degree of agglomeration of casein micelles and its chains in acid and lysozyme coagulate results from a similar mechanism of formation of both types of coagulate. The discussed similarities were also observed during the analysis of electronograms obtained under the scanning microscope.

The structure of lysozyme coagulate was to a large extent similar to that of acid curd of milk. Attention should be paid to the finer porous and spongy structure of rennet curd from milk modified with lysozyme, in comparison with rennet coagulate (photographs 5, 6, 7, 8).

The presented influence of lysozyme on rennet coagulation, the possibility of precipitating casein from milk by this enzyme and also the similarity of the structure of lysozyme and acid coagulate of casein from cow's milk make it possible to presume that the effect of lysozyme on casein is connected with the partial reduction of electrokinetic potential of casein micelles. These changes may also exert a certain influence of the digestion processes, a fact of practical significance in the nutrition of infants and children.

CONCLUSION

1. Lysozyme addition to milk shortened the time of rennet coagulation of casein.
2. The analysis of electronograms (TEM, SEM) revealed that the structure of lysozyme coagulate was similar to the acid curd structure of milk.
3. The reported influence of lysozyme on rennet coagulation and also the similarity of the structure of lysozyme and acid coagulates of casein from cow's milk make it possible to suppose that the effect of lysozyme on casein is connected with a partial reduction of the electrokinetic potential of casein micelles.

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WPLYW LIZOZYMU NA ENZYMATYCZNĄ KOAGULACJĘ MLEKA

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Streszczenie

W pracy prześledzono wpływ dodatku lizozymu (0,5; 1,0 i 2,0 mg/cm³) na czas koagulacji podpuszczkowej kazeiny. Badania te przeprowadzono na świeżym mleku odtłuszczonym, roztworze kazeiny w dializacie mleka i sztucznym serum mleka, a także zawieszynie miceli kazeinowych w dializacie oraz sztucznym roztworze soli mleka. Stwierdzono, że we wszystkich układach doświadczenia, lizozym skracał czas podpuszczkowej koagulacji kazeiny proporcjonalnie do jego dodatku (tab. 1). Nie zaobserwowano praktycznie żadnych różnic w podatności próbek kazeiny na działanie podpuszczki w zależności od czasu ich wstępnej inkubacji z lizozymem (tab. 2).

W dalszej części pracy określono strukturę koagulatu wytrąconego z mleka przez lizozym (12 mg/cm³), a także koagulatu podpuszczkowego mleka traktowanego lizozymem (2 mg/cm³). W celu porównania określono strukturę skrzepu podpuszczkowego i kwasowego mleka. Obserwacji koagulatów dokonano przy zastosowaniu mikroskopu elektronowego transmisyjnego (TEM) Tesla BS-500 oraz za pomocą mikroskopu elektronowego skaningowego (SEM) JEOL-JSM S1 (przy napięciu roboczym 10 MEV).

Analiza uzyskanych elektrogramów wykazała podobieństwo struktury koagulatu lizozymowego i kwasowego (zdjęcia 2, 4, 6, 8). Oba rodzaje skrzepów charakteryzowały się wysokim (w porównaniu z koagulatem podpuszczkowym) stopniem zaglomerowania cząsteczek kazeiny oraz jej łańcuchów. Większa aglomeracja kazeiny w skrzepie kwasowym związana jest prawdopodobnie z szybką i niemal całkowitą neutralizacją ładunków powierzchniowych miceli. Podobny stopień aglomeracji miceli kazeinowych w koagulacie kwasowym i lizozymowym wynikać może ze zbliżonego mechanizmu tworzenia się obu rodzajów skrzepu.

Wykazany w pracy wpływ lizozymu na koagulację podpuszczkową, możliwość wytrącania kazeiny z mleka przez ten enzym oraz podobieństwo struktury koagulatu lizozymowego i koagulatu kwasowego kazeiny mleka krowiego pozwalają przypuszczać, że oddziaływanie lizozymu na kazeinę łączy się z częściową redukcją potencjału elektrokinetycznego miceli kazeinowych. Zmiany te mogą wywierać pewien wpływ na procesy trawienia białek mleka, co może mieć istotne znaczenie praktyczne przy zastosowaniu lizozymu w humanizacji mleka krowiego.