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PRESSURE-JUMP KINETICS OF BOVINE β-CASEIN MICELLIZATION

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Two samples of highly purified bovine β-casein supplied to us by Dr. T.A.J. Payens of the National Institute for Dairy Research, Ede, The Netherlands, were studied over a range of concentration from just below the critical micelle concentration (CMC) to 0.46%, in 0.2 ionic strength phosphate buffer (pH 7.0), at 20 and 25 °C. The relaxation process studied by pressure jump using a 90° scattered light detector was also confirmed by the temperature-jump method. In the pressure-jump experiments, the process could be separated into two general time domains, with an approximate ratio of 10–25 : 1, a behavior reminiscent of that found for synthetic micellar systems. The faster relaxation process was still exhibited below the CMC, however. The concentration dependence of the faster relaxation time agreed very satisfactorily with predictions from the micelle model described in the companion paper.

1. Introduction

The pressure-jump light-scattering apparatus developed in this laboratory and previously described [1,2] was used to study the relaxation kinetics of two samples of bovine β-casein at 20 and 25 °C in 0.2 ionic strength (pH 7.0) phosphate buffer. The possibility of examining the kinetics of micellization with this method was already foreshadowed by the high-pressure light-scattering observations reported by Payens and Heremans [3], which were presented by Dr. Payens at a seminar at the University of Connecticut. The pressure-jump relaxation process could be separated into two general time domains, with an approximate ratio of 10–25 : 1. While cell recooling interfered with the evaluation of the slower time domain with the temperature-jump method, the faster time domain relaxation process was verified with a light-scattering temperature-jump apparatus which we had described previously [4,5].

2. Description of samples

Two samples of bovine β-casein were kindly supplied to us for this study by Dr. T.A.J. Payens of the National Institute for Dairy Research, Ede, The Netherlands. A brief description of the samples by Dr. Payens is reproduced here: - - - "two lyophilized specimens, prepared from the milk of individual cows. Both beta caseins have been phenotyped by starch gel electrophoresis and recognized as the genetic polymorph beta-A1. Beta casein A1 from cow 'Adje 166' has not been subjected to a final purification step of DEAE-

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chromatography. - - - Beta casein A1 from cow 'Josje 2' is highly purified. - - - Both caseins have been prepared by two members of our group: Miss Paula Both and Mr. B.W. van Markwijk. The latter also measured the concentration-dependence of the apparent molecular weight by light scattering." We are sincerely indebted to Dr. Payens and the members of his group for these samples and the accompanying data, which made this work possible. The apparent molecular weight data are shown for the highly purified Josje 2 sample in the companion paper [6], and their use in correlating the faster relaxation time behavior as a function of concentration is described in section 5 of the present paper.

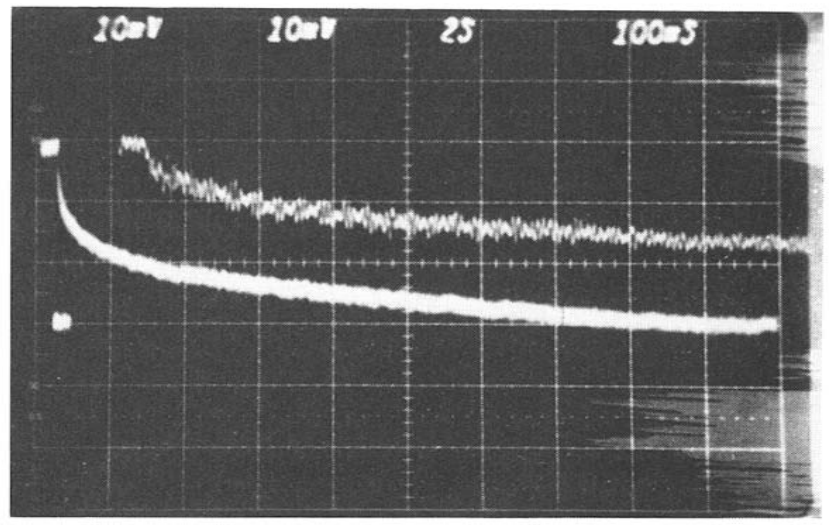

Fig. 1. Pressure-jump relaxation curve for 0.3248% β-casein sample Josje 2 in 0.2 *I* phosphate buffer (pH 7.0), at 20 °C. Upper trace, sweep rate 100 ms/division. Lower trace, sweep rate 2 s/division. Both traces began simultaneously, cf. data in fig. 3.

3. Experimental

Lyophilized preparations were stored in a refrigerator freezing comparment, and small samples were removed and dissolved directly into phosphate buffers without dialysis, as needed. The pressure-jump apparatus of our design starts with the sample at atmospheric pressure and raises the pressure to its final level in several milliseconds. The final pressure was varied systematically with a given cell filling, to ascertain that there was no dependence of the observed relaxation time on pressure. A Tektronix model 5444 oscilloscope with a type 5B44 dual time base containing two independent beams and two separate horizontal sweeps was used to record the kinetics, photographs being taken with a model C-5A Polaroid camera with open shutter. Light transmitted through the cell was attenuated with a polarizer-analyzer combination and received by an EMI type 9558 QB front window photomultiplier. The output from this photomultiplier was subtracted from that of a similar photomultiplier receiving light scattered from the sample at 90°, using a Tektronix Model AM502 differential amplifer. The difference signal was connected to the two independent vertical amplifiers of the oscilloscope in parallel. This made it possible to record both the faster and slower time domains in each experiment with high resolution. Further details of the apparatus are provided in the cited references [1,2]. The photographs were mounted in a David Mann 2-coordinate microcomparator, and 50–100 data points of scattered light amplitude vs. time were routinely read, and recorded manually. These primary data were then entered into appropriate computer programs, described below, to be processed in the University of Connecticut IBM 370 computer. A photograph of two time domains from a pressure-jump relaxation experiment on a 0.3248% sample of Josje 2 at 20 °C is shown in fig. 1. Fig. 2 shows the results of a temperature-jump experiment on a 0.339% sam-

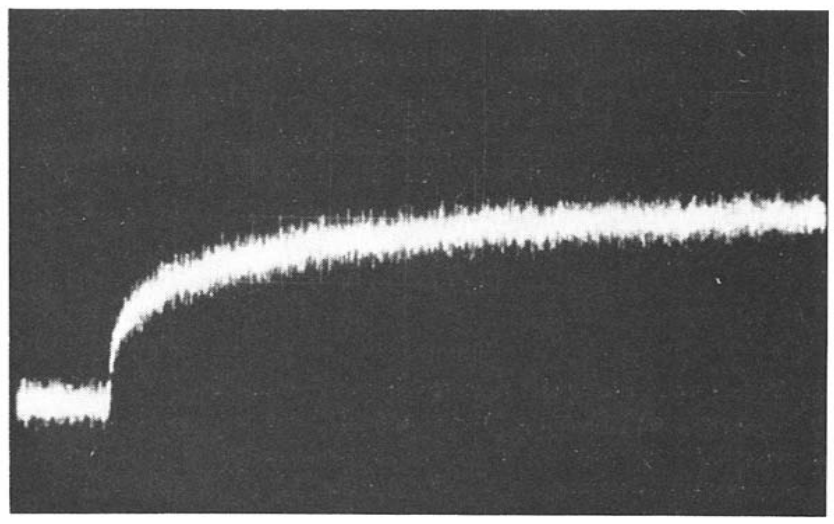

Fig. 2. Temperature-jump relaxation curve for 0.339% β-casein sample Adje 166 in 0.2 *I* phosphate buffer (pH 7.0). Sweep rate 50 ms/division. Heating time constant ($RC/2$) 25 μs. Final temperature 24 °C. cf. data in fig. 6.

ple of Adje 166 at a final temperature of 24°C in the fast time domain.

4. Treatment of data

As tabulated in detail for each photograph evaluated [7], amplitudes and reciprocal relaxation times were extracted for two time domains, at all concentrations above the CMC, or critical micelle concentration. Mathematical procedures for separation of two time domains have been described in independent developments by Johnson and Schuster [8] and by Provencher [9,10], in his program called 'DISCRETE'. We are indebted to the authors of both programs for making them available to us. In table 1 is shown a comparison between the time analyses by these two methods for five experiments. The method of Johnson and Schuster is most advantageous when a very large number of data points, such as 1000, are available, furnished by digital output from a waveform storage recorder. Since it was prohibitive to read much more than 100 points under the microcomparator, it was found more suitable to use the Provencher DISCRETE computer program for general evaluation of experiments. Experiments such as that shown in fig. 1 were repeated several times for each cell filling, and these covered a wide range of concentration at two temperatures, 20 and 25°C. Table 1 illustrates the rather wide scatter in replication of experiments at 0.1% concentration, under identical conditions. In addition, a number of different final pressures were selected, from 43 to 73 atm., in order to study whether any systematic nonlinearity in response was being introduced by excessive pressure perturbation of the starting equilibrium condition. Detailed examination of results at these different final pressures did not indicate any nonlinearity effects, within experimental error [7]. The results at different pressures are therefore all plotted together, when correlated with predictions from the shell model, as discussed below.

5. Correlation of reciprocal relaxation times

In a previous publication [11], the kinetic consequences were worked out for the initial relaxation period, based on the shell model for thermodynamically ideal micelle solutions [12]. Since the companion paper [6] shows that this model describes reasonably well the molecular weight and size distribution and the sedimentation behavior of β-casein micellar systems, these predictions are now used to correlate the experimental kinetics results. The complete relationship between the initial reciprocal relaxation time $1/\tau$ and the concentration is given [11] by

$$1/\tau = (k_f/K[A_0])\{C^0 + [A_0](fK[A_0] - 1)\}/(1 - M/M_w) \quad (1)$$

where k_f is the intrinsic forward rate constant, K the corresponding intrinsic formation constant per

Table 1

Comparison of relaxation time between two methods

Conditions: β-Casein Adje 166 in phosphate buffer at pH 7.0, $I = 0.2$, $t = 20°C$, $\Delta P = 73.88$ atm. Values of $1/\tau_s$ and $1/\tau_f$ are expressed as s^{-1}.

Method				Number of points	Concentration ($\bar{c}$)
Johnson and Schuster [8]		Provencher [9,10]			
$1/\tau_s$	$1/\tau_f$	$1/\tau_s$	$1/\tau_f$		
0.1304	1.7807	0.1145	1.492	23	0.10
0.1457	1.5123	0.1701	1.933	24	0.20
0.0992	1.2238	0.0842	1.190	162	0.10
0.1634	1.5039	0.1689	1.539	129	0.10
0.1479	1.4321	0.1426	1.646	100	0.10

monomer-micelle bond, $[A_0]$ the free monomer concentration, C^0 the total equivalent concentration of β-casein, in terms of an assumed monomer molecular weight, M, of 24000, M_w the weight-average molecular weight, and f the (anti) nucleation factor of the shell model [12], suppressing the monomer-monomer interaction. At each experimental concentration, the value M/M_w was taken from the appropriate experimental determinations of B.W. van Markwijk, one of which is shown in the companion paper [6] for the highly purified Josje 2 sample of β-casein. The values of the monomer concentration $[A_0]$ and of the intrinsic formation constant K required to predict the correct total equivalent concentration C^0 are determined by the appropriate choices for the nucleation factor f, the maximum possible number, n of monomers added to one original target monomer, and by the equivalent concentration at the critical micelle concentration, CMC, as explained in the companion paper [6]. Since $fK[A_0] \ll 1$ for most conditions, once f, n and K are determined, a linear plot vs. $C^0/[A_0]-1$ may be made, according to

$$(1/\tau)(1 - M/M_w) \approx k_b(C^0/[A_0]-1) \qquad (2)$$

A least-squares plot is made, using experimental values for $1/\tau$, and $[A_0]$ values predicted as indicated above. The slope of this plot provides the intrinsic rate constant for dissociation, k_b. Where data are available in the literature [13], at 20°C, the experimental value is used for the CMC. Under different conditions of temperature (25°C), the CMC value is estimated from a series of trial and error plots just described. These plots pass through the origin for the correct choice made for the CMC.

6. Results

The results for the initial reciprocal relaxation time are shown in the form in which they are obtained experimentally, as a direct plot against the total weight concentration. Since all the pertinent parameters are now evaluated, the theoretical prediction from eq. 1 is also shown as the solid curve. In figs. 3–6 are shown the results for both β-casein samples, at the two different temperatures.

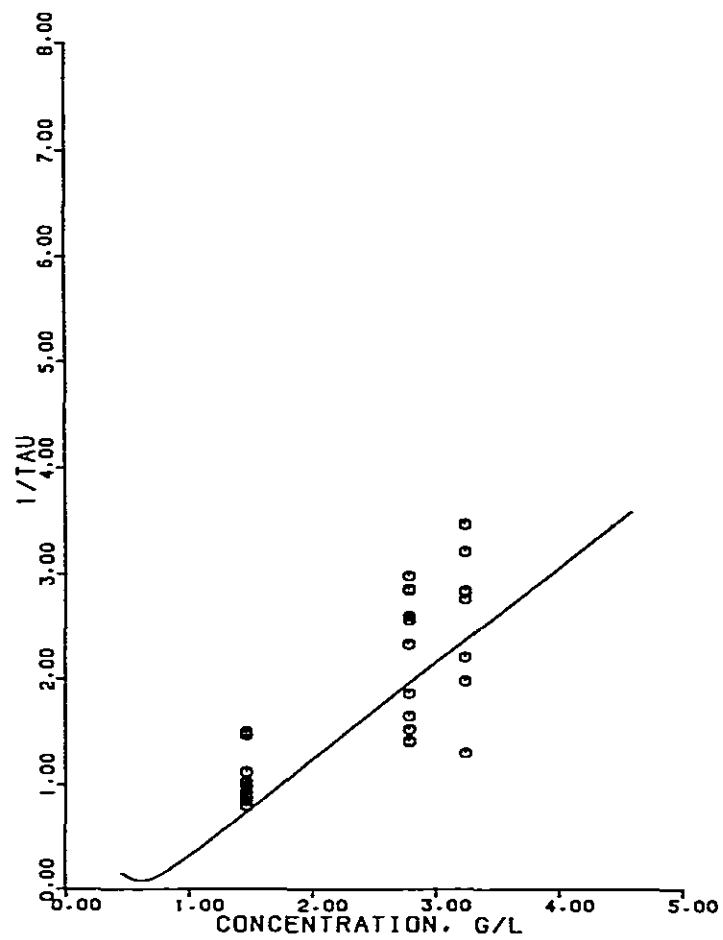

Fig. 3. Concentration dependence of the reciprocal relaxation time (fast process) of β-casein sample Josje 2 at 20°C, in phosphate buffer (pH 7.0), $I = 0.2$. Points are as measured by pressure-jump experiments (total 30 points). (———) Calculated from the shell model for $n = 46$, $f = 2\times10^{-4}$, CMC = 2.55×10^{-5} M.

7. The slow relaxation time

While the reciprocal relaxation time for the slow time domain and the corresponding amplitude have been obtained for each experiment and are tabulated [7], the micelle model being used now does not offer a closed-form prediction for a steady-state value for the slow relaxation. According to the theory of Aniansson and Wall [14,15], which assumes a Gaussian distribution of micelles, this process corresponds to the complete dissolution of micelles. They offer a closed-form solution

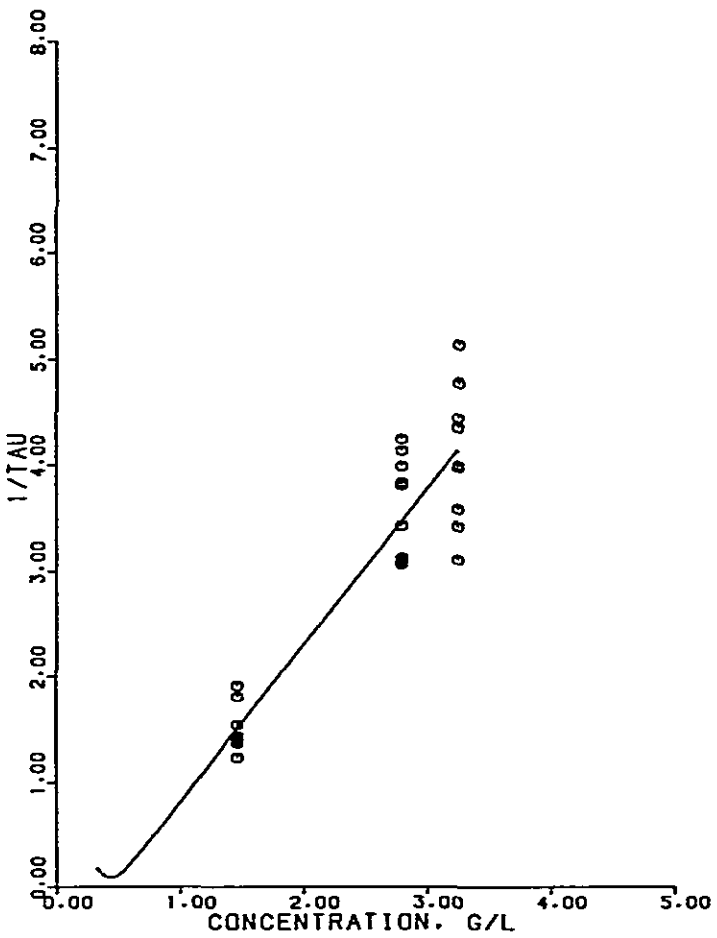

Fig. 4. Concentration dependence of the reciprocal relaxation time (fast process) of β-casein sample Josje 2 at 25 °C, in phosphate buffer (pH 7.0), $I = 0.2$. Points are as measured by pressure-jump experiments (total 27 points). (———) Calculated from the shell model for $n = 46$, $f = 2 \times 10^{-4}$ M, CMC = 1.80×10^{-5} M.

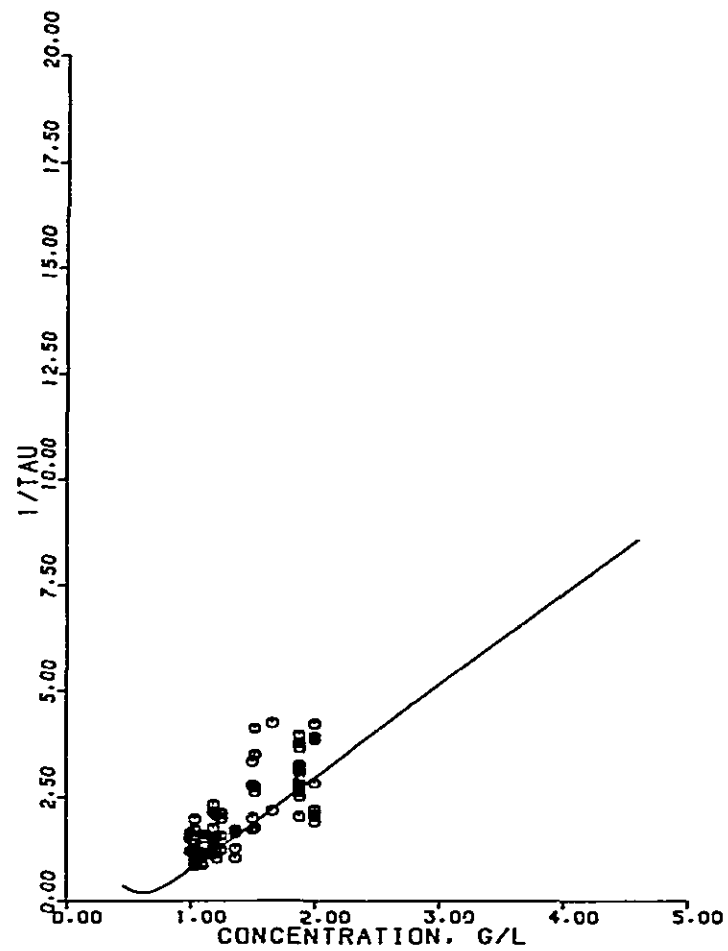

Fig. 5. Concentration dependence of the reciprocal relaxation time (fast process) of β-casein sample Adje 166 at 20 °C, in phosphate buffer (pH 7.0), $I = 0.2$. Points are as measured by pressure-jump experiments (total 79 points). (———) Calculated from the shell model for $n = 46$, $f = 2 \times 10^{-4}$, CMC = 2.55×10^{-5} M.

for its evaluation. It is possible to compute the rate of micelle dissolution directly from the shell micelle model, which describes the equilibrium properties of β-casein fairly well [6]. Very lengthy computations are required, a separate one for each starting concentration, to predict the rate of dissolution after other superposed processes have all been finally damped out and the effect of dissolution alone is uncovered. While this has been done in several cases [16], it has not yet been done systematically to correspond to the experimental conditions covered in this study. If that turns out to be feasible, it may be the subject for a future communication.

8. Concentrations below the CMC

One such experiment has been performed [7] for the sample Josje 2 at 20 °C. This shows that the faster relaxation process is still present, as predicted [11], while the slower one has disappeared. A detailed study of myosin fibrils below the CMC has been reported by Halvorson [17]. These results are consistent with predictions from the shell model [11,12].

9. Summary

Pressure-jump relaxation measurements using light-scattering detection have been performed on

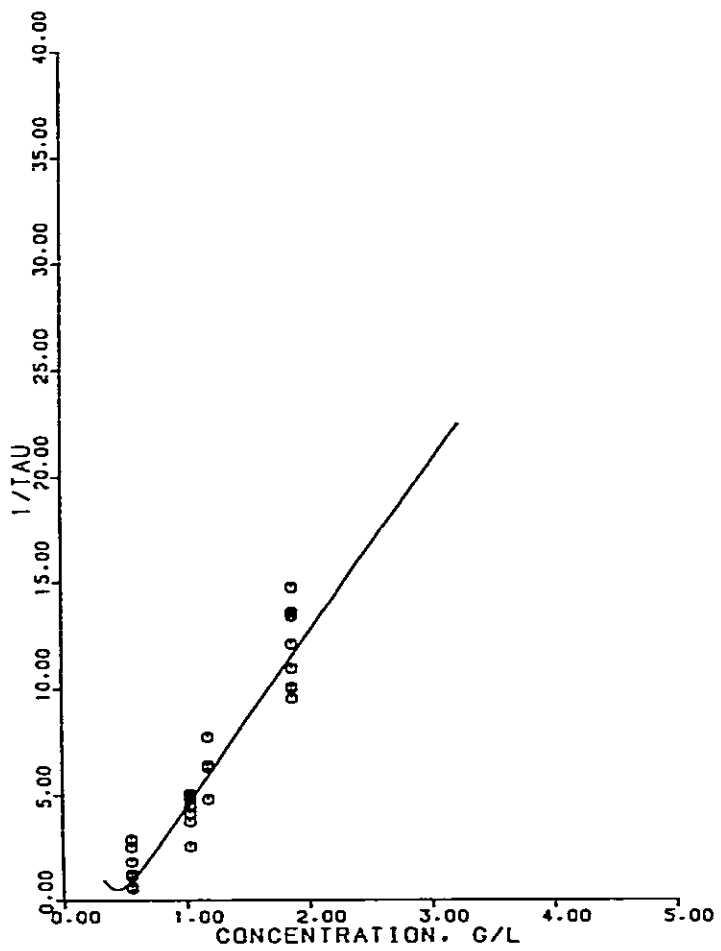

Fig. 6. Concentration dependence of the reciprocal relaxation time (fast process) of β-casein sample Adje 166 at 25 °C, in phosphate buffer (pH 7.0). $I = 0.2$. Points are as measured by pressure-jump experiments (total 31 points). (———) Calculated from the shell model for $n = 46$, $f = 2 \times 10^{-4}$ M, CMC = 1.80×10^{-5} M.

two samples of purified β-casein at 20 and 25 °C. At concentrations above the CMC, the relaxation takes place in two separate time domains, having a ratio of approx. 10–15 : 1. Both reciprocal relaxation times and their corresponding amplitudes have been tabulated [7]. For the initial relaxation process, satisfactory comparison is obtained between experiments and predictions based on the shell model for thermodynamic ally ideal micellar solutions [11,12].

Acknowledgments

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