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# KINETICS OF AGGREGATION OF $\alpha_{s1}$-CASEIN/$Ca^{2+}$ MIXTURES: CHARGE AND TEMPERATURE EFFECTS

D.G. DALGLEISH, E. PATERSON [1] and D.S. HORNE
*The Hannah Research Institute, Ayr KA6 5HL, Scotland, UK*

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Time-dependent light-scattering studies have been made on mixtures of $\alpha_{s1}$-casein and $Ca^{2+}$, at fixed temperature over a range of [$Ca^{2+}$] and [$\alpha_{s1}$-casein], and also as functions of temperature. Measurements were also made of the extent of precipitate formation in the casein/$Ca^{2+}$ mixtures, using centrifugation. The results are analysed in terms of a monomer–octamer equilibrium between calcium caseinate particles followed by a Smoluchowski aggregation in which only the octamers can participate. The equilibrium constant is dependent upon the charge on the protein/$Ca^{2+}$ particles, and hence can be related to the extent of binding of $Ca^{2+}$ to the $\alpha_{s1}$-casein. The Smoluchowski constant is likewise shown to be charge-dependent. The variation of the reaction rate with temperature can be ascribed solely to the changing charge of the $\alpha_{s1}$-casein/$Ca^{2+}$ complex caused by changed binding of $Ca^{2+}$ at different temperatures.

## 1. Introduction

We have previously reported on the kinetics of aggregation of bovine $\alpha_{s1}$-casein caused by calcium ions [1,2]. These studies showed that the time-course of molecular weight growth during the aggregation apparently involves two stages, the first consisting of a slow but increasing rate of growth of weight-average molecular weight ($\bar{M}_w$) and the second being a linear growth of $\bar{M}_w$, according to the mechanism of von Smoluchowski [3]. Not all of the $\alpha_{s1}$-casein is active in this second stage, and other reports have shown that indeed not all of the casein is precipitable in the range of concentrations of calcium ions which we have used (up to 10 mM $Ca^{2+}$) [4,5]. The major factor governing the rate of precipitation of the $\alpha_{s1}$-casein is the charge carried by the casein-$Ca^{2+}$ complex [2]. The first process in the overall reaction leading to precipitation is the binding of $Ca^{2+}$ to the casein: at pH values around neutrality, $\alpha_{s1}$-casein possesses a net negative charge of some 22 units [6], which is progressively reduced by the binding of increasing numbers of the positively-charged $Ca^{2+}$. When some 7–8 ions of calcium have been bound to each $\alpha_{s1}$-casein molecule reducing the net charge on the monomer to about −6 units, precipitation of the protein is initiated, and we have suggested that, at about this point, repulsive forces between the negatively charged casein monomers are inadequate to prevent coagulation. It was demonstrated that the rate of aggregation during the linear portion of the molecular-weight growth curve was proportional to the negative exponential of a constant times the square of the monomer charge, which is determined in its turn by the extent of calcium binding.

The present investigation was intended to make a full study of the aggregation reaction involving $\alpha_{s1}$-casein and $Ca^{2+}$, in a further attempt to establish the detailed mechanism of the reaction. Much of the previous work was based on the analysis of turbidimetric measurements, which are not readily translatable into molecular weights. Also, we had previously not considered the first stage of the reaction in any detail, nor had the extent of precipitation and its influence on the reaction been explicitly considered. Additionally, it is known that the reactions of caseins are very temperature-dependent, as evidenced by the high activation energy of milk protein being precipitated by heat [7], the fact that renneted micelles aggregate fast at 20 °C and not at all at 10 °C [8], and the

[1] Present address: Research Laboratory, Arthur Guinness Son & Co., Park Royal Brewery, London.

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change in aggregation of β-casein with temperature [14,15]. We have studied this temperature-dependence of the kinetics of $\alpha_{s1}$-casein aggregation with the view of explaining the apparent anomaly that a reaction which is fast at room temperature yet has a very high apparent activation energy.

## 2. Experimental

Preparation of pure bovine $\alpha_{s1}$-casein has been described previously [9,10]. In all of the experiments described, $\alpha_{s1}$-casein and $Ca^{2+}$ were prepared in a buffer of 0.02M imidazole/HCl, pH 7.0 which also contained 0.05M NaCl. "Analar" grade $CaCl_2 \cdot 6H_2O$ (BDH Ltd) was used as the source of $Ca^{2+}$, concentrations of which were standardized by titration with EDTA (Sigma Chemical Co., Poole), using a calcium ion-sensitive electrode (Radiometer Type F2112 Ca, Copenhagen) as the end-point indicator.

The use of a stopped-flow reaction system for determining fast changes in light-scattering during a reaction has already been described [1]. No major differences between the present and earlier systems exist: an extra 48 channels of correlator store were incorporated, thus extending the maximum time-scale to 96 seconds, and also the reaction system was jacketed to allow circulation of thermostatted water. The latter modification allowed adjustment of the reaction temperatures to be made: temperatures within the reaction cell were measured by a calibrated thermistor built into the flow system. In earlier experiments [1] reactions were measured to relatively high extents of reaction and to very large molecular weights. The present study sought to clarify the earlier stages of the reaction, and so the investigations were made over relatively shorter time-scales.

Measurements were made of the time-course of molecular-weight growth, for six different $\alpha_{s1}$-casein concentrations (0.25, 0.5, 0.75, 1.0, 1.5 and 2 mg/ml) and six concentrations of $Ca^{2+}$ (0.0065 M to 0.009 M in steps of 0.0005 M), giving 36 casein/$Ca^{2+}$ combinations. In practice, combinations of 0.25 mg/ml casein with $[Ca^{2+}] < 0.008$M, and 0.5 and 0.75 mg/ml casein with 0.0065 M $Ca^{2+}$ gave reactions which were too slow to measure conveniently. Measurements of $\bar{M}_w$ as functions of time after the initial mixing of the $\alpha_{s1}$-casein and $Ca^{2+}$ solutions were made for all other

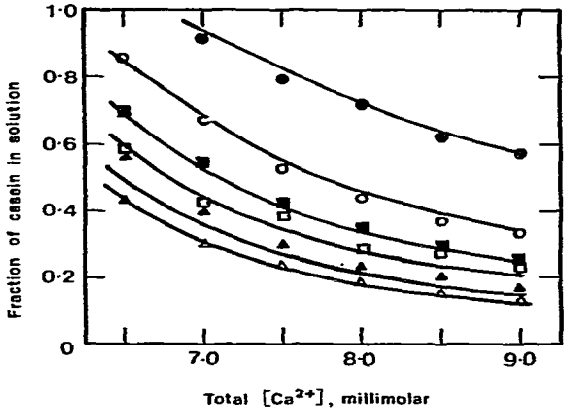


Fig. 1. Extent of precipitation of $\alpha_{s1}$-casein as a function of the total concentration of $Ca^{2+}$. The curves shown are, moving upwards from the lowest curve, measurements made at concentrations of 2, 1.5, 1.0, 0.75, 0.5 and 0.25 mg/ml of $\alpha_{s1}$-casein. Solid lines represent the fits to the experimental observations calculated by the equilibrium described in the text.

casein/$Ca^{2+}$ combinations, at a temperature of 23°C. The temperature-dependence of the reactions was studied using mixtures containing 1 mg/ml of $\alpha_{s1}$-casein and 0.007 M $Ca^{2+}$, in the range 17°–30°C. These temperature limits were imposed by the reaction itself: at the lower end of the range the reaction is too slow and at the upper end too fast to measure with the existing equipment.

In parallel with the studies of kinetics at 23°C, the fraction of casein which precipitated from each of the casein/$Ca^{2+}$ mixtures was measured. The mixtures were made up, and were incubated in a water bath at 23°C for 1 hour. The solutions were then centrifuged at 40 000 g for 2 hours at 23°C (MSE Superspeed 65, 6 × 38 ml swing out rotor). The supernatant solution was removed and its absorbance was measured, to determine the concentration of unprecipitated $\alpha_{s1}$-casein ($E^{1\%}_{1cm} = 10$) (ref. [11]). These results were used in defining the equilibria described in the next section.

## 3. Results

### 3.1. Extent of casein precipitation

Fig. 1 shows the results of the experiments which

determined the amount of unprecipitated casein in each of the casein/$Ca^{2+}$ combinations. In none of the mixtures was the $\alpha_{s1}$-casein completely precipitated: appreciable amounts, in some cases exceeding 50% of the $\alpha_{s1}$-casein, remained soluble. The extent of precipitation was seen to depend upon the concentrations of both $\alpha_{s1}$-casein and of $Ca^{2+}$, such that an increase in either constituent increased the amount of precipitate formed. It was clear that an equilibrium was established between precipitated and non-precipitated casein in all cases, within the time of the experiment. Incubation of the mixtures for longer times prior to centrifugation did not increase the extent of precipitation. Thus, there appears to be a $Ca^{2+}$-dependent equilibrium between soluble and precipitable caseins, and the differences between the different mixtures did not arise from differences in the rates of aggregation.

In view of the previous finding [2] that the rate of aggregation in $\alpha_{s1}$-casein–$Ca^{2+}$ mixtures is dependent on the charge on the protein–$Ca^{2+}$ complex, we sought to explain the amount of precipitate formed in terms of equilibria between differently-charged casein/$Ca^{2+}$ complexes. The extent of binding of $Ca^{2+}$ to $\alpha_{s1}$-casein [12,13] is known, so that it is possible to investigate the effect of the alteration of protein charge, caused by changing $[Ca^{2+}]$, on the precipitation equilibria.

From the conditions of centrifugation, the material forming the precipitate must have a sedimentation coefficient of about 120 S, corresponding to a molecular weight of some millions, that is, a degree of aggregation of some hundreds. This is in equilibrium with lower molecular weights aggregates, or indeed possibly with monomer. Kinetic studies (see later sections) show that the final production of precipitate is a Smoluchowski reaction, in which all aggregating material participates. If the kinetic and the equilibrium properties of the reaction are considered together, it is necessary to postulate that monomers do not aggregate directly to form precipitate, but must initially form a limited subaggregate. Formation of such a subaggregate can be considered as a micellisation reaction, i.e,

$$n\mathrm{M} \rightleftharpoons \mathrm{M}_n\ , \quad K_n = [\mathrm{M}_n]/[\mathrm{M}]^n\ , \qquad (1)$$

where M represents a monomeric $\alpha_{s1}$-casein–$Ca^{2+}$ unit, and $n$ is an integer number. $M_n$ then is the aggregating unit which forms the final precipitate. In such micellisation reactions there is a concentration of monomer below which virtually no $M_n$ is formed, and above which the formation of $M_n$ is possible. If sufficient monomer is initially present to allow the formation of micelles, and then the micelles are removed (by e.g. precipitation or centrifugation) this will leave a concentration of monomer which is capable of forming only very small amounts of new micelles, since it is only slightly higher than the critical micelle concentration. This is in accord with the results in fig. 1, and thus we consider the formation of $n$-mer to be the first stage in the reaction. Subsequent aggregation of $n$-mer then produces precipitate.

The concentration of unprecipitated casein, and the initial casein concentration allow the calculation of $K_n$ for any value of $n$, and it remains to determine the best value of $n$ which will fit the experimental determinations. From previous results [2], we expect

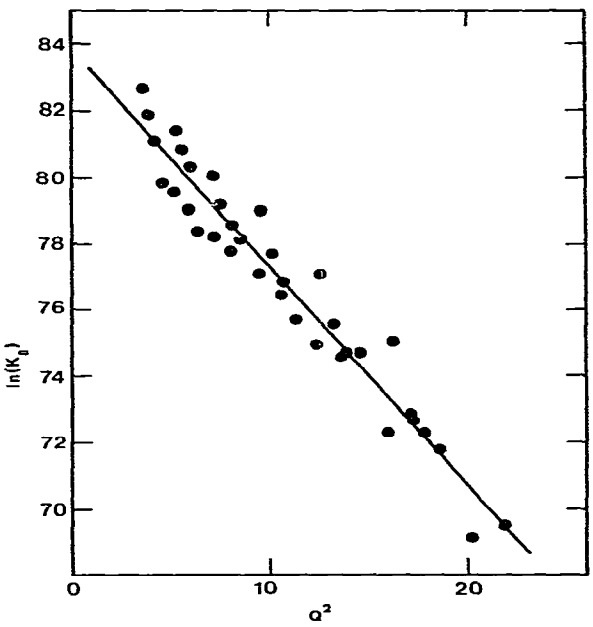


Fig. 2. Dependence of the equilibrium constant $K_s$ upon protein charge, for different $\alpha_{s1}$-casein/$Ca^{2+}$ mixtures. Individual values of $K_s$ were calculated from the data in fig. 1. $Q^2$ was calculated from the binding isotherm.

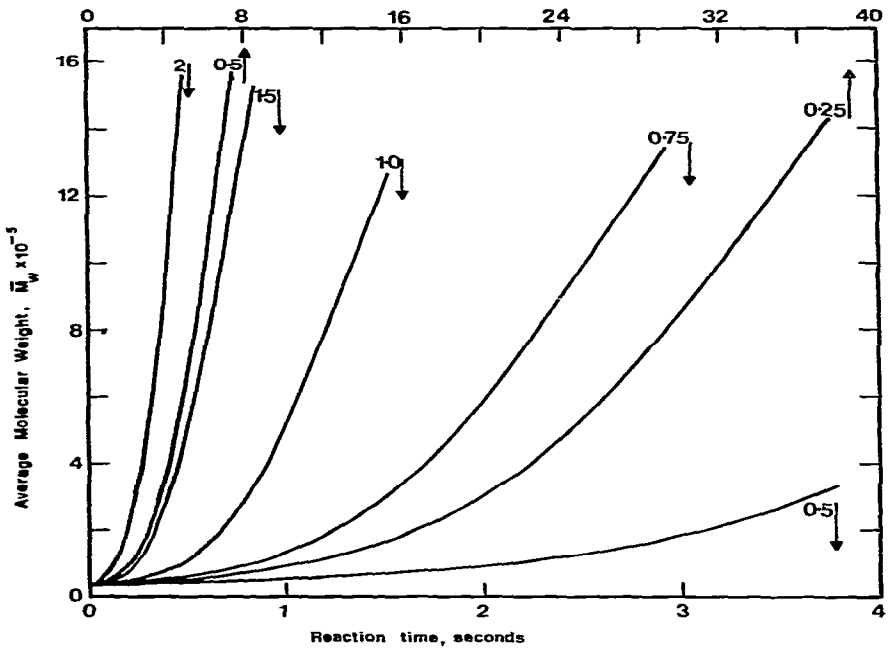


Fig. 3. Growth of molecular weight with time after mixing of solutions of $\alpha_{s1}$-casein/$Ca^{2+}$. Total concentration of $Ca^{2+}$ was 0.009 M for all experiments. Numbers on the curves define the casein concentration in mg/ml. Arrows refer to the different time-scales at top and bottom of the figure. Results for 0.5 mg/ml protein are plotted on both scales. Temperature was 23 °C.

$K_n$ to have the form:

$$K_n = a_n \exp(-b_n Q^2) , \quad (2)$$

where $Q$ is the charge number on the casein—$Ca^{2+}$ monomers, and $a_n$ and $b_n$ are constants. Thus, for any chosen value of $n$ a plot of ln $K_n$ against $Q^2$ should be linear. This was found to be so, for any selected concentration of $\alpha_{s1}$-casein and varying $[Ca^{2+}]$. However, when plots for different casein concentrations were compared, they were found to depend upon casein concentration, to a greater or lesser extent, depending on the selected value of $n$ (i.e. $K_n$ did not represent a true equilibrium constant at these values of $n$). At values of $n$ close to 8, the plots became concentration-independent, and the following calculation was used to define the best value of $n$ which gave a concentration-independent value of $K_n$. For all of the 36 mixtures of casein and $Ca^{2+}$, $Q^2$ and $K_n$ were calculated, for all values of $n$ from 2 to 16. This information was used to calculate the regression line of ln $K_n$ against $Q^2$ for each value of $n$, and, in particular, the standard deviation of the points from the best line was calculated. This deviation was high at $n = 2$, decreased to a minimum at $n = 8$, and then increased for $n > 8$. Therefore the best fit of all the experimental points was found to be for $n = 8$, giving a charge-dependent and concentration-independent set of constants $K_8$. It was therefore taken that the formation of precipitate was governed by the equilibrium:

$$8M \rightleftharpoons M_8 . \quad (3)$$

This relation between $K_8$ and $Q^2$ is shown in fig. 2. The slope of the plot allows $K_8$ to be related to charge by

$$\ln(K_8) = 83.9 - 195Q^2/T . \quad (4)$$

The equilibrium constants can then be used to fit the data of fig. 1, as shown in that figure.

This monomer—octamer equilibrium may then be taken as the starting point of the Smoluchowski pre-

cipitation reaction, and thus infinite aggregation is possible only for the octamer units. The formation of "micellar" aggregates of $\beta$- and $\kappa$-caseins is already known [14–17], and it seems that the $\alpha_{s1}$-casein–$Ca^{2+}$ complexes show this tendency also, although $\alpha_{s1}$-casein in the absence of $Ca^{2+}$ shows only a limited tendency to form aggregates [24].

### 3.2. Kinetics of the aggregation

The time-course of molecular weight growth can be characterized by a lag-stage, where the rate of growth of molecular weight is slow but increasing, and a second stage where molecular weight growth is linear with time [1,2]. The length of the lag-stage and the gradient of the linear stage depend upon the concentrations of both casein and $Ca^{2+}$. Fig. 3 shows the effect of changing casein concentration at a fixed concentration of $Ca^{2+}$. As the concentration of casein is decreased, the rate of molecular weight growth decreases markedly. Comparison of results obtained from all casein/$Ca^{2+}$ combinations at 23°C confirmed and extended the finding from turbidity measurements [2] that all the reaction profiles may be superimposed if molecular weight is plotted against reduced time, $t/t_c$, where $t_c$ is the "critical time", found by extrapolating the linear portion of the curve back to monomer molecular weight. It had previously been shown that this procedure allowed superimposition of the reaction profiles at a constant casein concentration and variable [$Ca^{2+}$]. This applies also to results at fixed [$Ca^{2+}$] and changing casein concentration. Additionally, it is not necessary to define reduced time as $t/t_c$: $t_c$ may be replaced by the time required for the molecular weight to attain any specific value. The composite curve on a time-scale reduced by the time taken to give $\bar{M}_w = 10^6$ is shown in fig. 4. The fact that the reaction profiles are superimposable in this way is to be taken as an indication that both phases of the reaction are controlled by similar factors. Indeed, it can be shown that, at a fixed temperature, the slope of the linear stage and the critical time are inversely related to one another.

### 3.3. The linear-growth stage of the reaction

We have shown earlier [1] that the linear growth of $\bar{M}_w$ with time is dependent on the amount of pre-

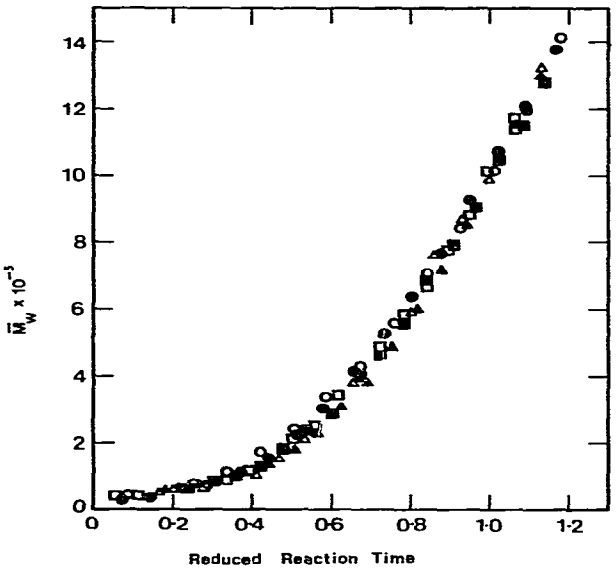


Fig. 4. Molecular weight as a function of reduced time for the reaction: reduced time is defined as the actual time divided by the time to attain $\bar{M}_w$ of $10^6$. Points are shown for 0.009M $Ca^{2+}$ and casein concentrations of ○, 2 mg/ml, ●, 1.5 mg/ml; □, 1.0 mg/ml; ■, 0.75 mg/ml; △, 0.50 mg/ml; ▲, 0.25 mg/ml Temperature was 23 °C.

cipitate formed by a given casein/$Ca^{2+}$ combination. The linear growth of $\bar{M}_w$ occurs via a Smoluchowski-type reaction, giving

$$\bar{M}_w(\text{agg}) = M_0(1 + 2k_s c t) , \qquad (5)$$

where $M_0$ is the molecular weight of the aggregating units, $k_s$ is a rate-constant, and $c$ is the molar concentration of aggregating material. This relationship does not take account of material which cannot aggregate, but is nonetheless present in the system. In such a case, the overall molecular weight is given by:

$$\bar{M}_w = w_{agg}\bar{M}_w(\text{agg}) + w_{\text{non-agg}}\bar{M}_w(\text{non-agg}) , \qquad (6)$$

where the $w$ factors represent the weight-fractions. During the linear-growth stage, there is no change in $w_{agg}$ (i.e. the equilibrium (3) is virtually complete): similarly $w_{\text{non-agg}}$ and $\bar{M}_w$(non-agg) do not change with time during this stage. Thus

$$d\bar{M}_w/dt = w_{agg}\, d\bar{M}_w(\text{agg})/dt = w_{agg}\, 2k_s c M_0 .$$

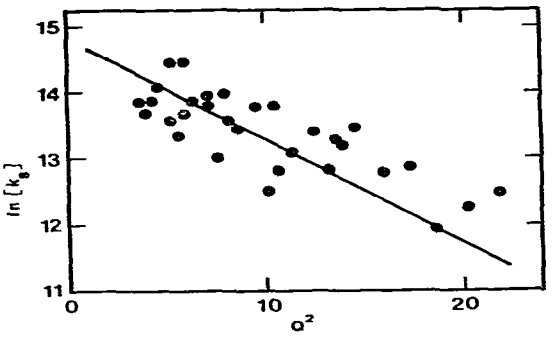


Fig. 5. Dependence of the Smoluchowski constant, $k_S$ upon protein charge at 23°C. $k_S$ was calculated from the measured $d\bar{M}_w/dt$ and precipitate concentrations. Charge was calculated from the binding isotherm.

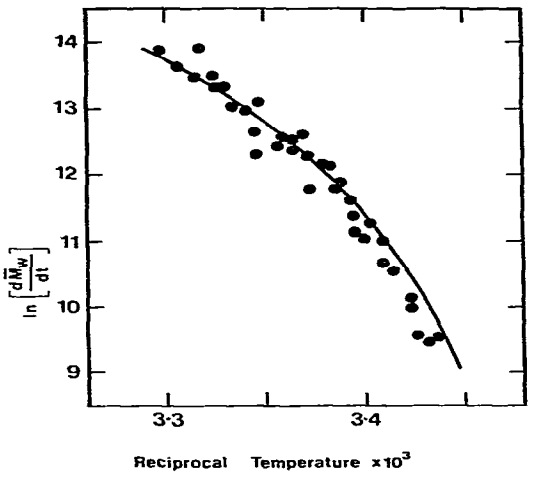


Fig. 6. Temperature-dependence of the precipitation, shown as an Arrhenius plot. The points represent individual measurements of $d\bar{M}_w/dt$ at different temperatures for 0.007 M $Ca^{2+}$ and 1 mg/ml of $\alpha_{S1}$-casein. The solid line represents the calculated value of $d\bar{M}_w/dt$ using the model based on charge interactions, as described in the text.

But $w_{agg} = nc/c_0$ where $c_0$ is the original molar monomer concentration, so that

$$d\bar{M}_w/dt = 2nM_0k_sc^2/c_0 \ . \qquad (7)$$

This expression takes into account the non-aggregated material, and thus differs from that which was used previously [1]. Experimentally, we know $d\bar{M}_w/dt$, $c_0$ and $c$, and from the equilibrium, $n = 8$ and $M_0$ is 8 times the monomer molecular weight of casein. Thus $k_S$ can be calculated according to eq. (7) for all of the experiments. When this was done, it was found that the calculated values of $k_S$ were not constant, but depended on the concentration of $Ca^{2+}$, and hence on the charge, $Q$, of the complexes. This variation is shown in fig. 5. Analysis of the plot yields the relationship

$$\ln(k_S) = 14.8 - 45.6\,Q^2/T \ . \qquad (8)$$

The linear portion of the molecular-weight growth is therefore defined by two factors, the aggregation rate constant $k_S$ and the concentration of aggregable material, $c$. Both of these are dependent upon the charge of the protein–$Ca^{2+}$ complex formed initially in a fast equilibration step. $k_S$ is directly altered by changing $Q^2$, and $c$ is formed by the equilibrium (3), whose equilibrium constant has been shown also to be charge-dependent.

### 3.4. The lag-stage of the reaction

The lag-stage of the reaction involves the formation of $M_8$ from monomer, and this stage of the reaction therefore involves two processes, namely the formation of $M_8$ and its subsequent aggregation, while the concentration of $M_8$ is increasing towards its equilibrium value. In attempting to analyse this stage, it must be recognised that the overall equilibrium (3) cannot be described so simply when the kinetics of the reaction are considered, since formation of octamer directly from monomer is a highly improbable event. The kinetic description of the formation of $M_8$, should thus ideally involve all possible intermediate species $M_2 - M_7$, in a series of bimolecular reactions. Analysis of the formation of $M_8$ in this way must therefore involve the many rate-constants required to describe the process. Once any $M_8$ is formed, its aggregation can be described by a modified Smoluchowski mechanism, which takes into account changes in the amount of material in the aggregating state [19,18]. We made calculations based on various multi-step models for forming $M_8$ which showed that the general form of the lag-stage can be predicted correctly. However, in view of the numbers of variables involved (forward and reverse rate-constants for every intermediate equilibrium step), it is not possible to define values for individual rate-constants in this stage of the reaction.

It was possible to demonstrate by such calculations

that the rate-determining step in the equilibration has to be the initial dimerization: it was necessary to keep the rate-constant for this step much lower than for subsequent steps. Also it was found that all of the stages of the reaction were required to be charge dependent, as is to be expected from the overall equilibrium constant $K_8$. This view of the lag-stage as an equilibration process which produces aggregable material supplants the theory expressed earlier [1] that the lag-stage represents the increasing rate constant for aggregation as particle size increases. The difference between the two descriptions is that the earlier attempt to explain the reaction did not take explicit account of the manner in which precipitable and non-precipitable material could be formed.

### 3.5. *Temperature-dependence of the reaction*

As the temperature of the reaction is increased, so the rate of precipitate formation is also increased. Fig. 6 shows the change in limiting $d\bar{M}_w/dt$ with temperature for mixtures containing 1mg/ml of $\alpha_{s1}$-casein and 7mM $Ca^{2+}$. The results are shown as an Arrhenius plot, demonstrating that it is not possible to describe the temperature-dependence of the precipitation reaction in terms of a single activation-energy-type process. Apparent activation energies derived from the gradients to the curve are as high as 100 kJ $mole^{-1}$, which are impossibly high for reaction such as the precipitation, which occurs readily at room temperature.

This temperature-dependence can be understood in terms of the processes described in the preceding sections. $d\bar{M}_w/dt$ is dependent upon the amount of precipitable material and also upon $k_s$, both which have been shown to depend on the charge of the $\alpha_{s1}$-casein/$Ca^{2+}$ complex. The charge on the casein–$Ca^{2+}$ monomer in turn depends on the number of $Ca^{2+}$ binding per casein ($\bar{\nu}$), which partially neutralize the initial negative charge on the protein. The binding of $Ca^{2+}$ to $\alpha_{s1}$-casein has been shown to be temperature-dependent [13]. Thus, since the overall concentrations of casein and $Ca^{2+}$ are unchanged during the experiments, $\bar{\nu}$ and hence $Q$ will vary with temperature. From the published information on the temperature-dependence of binding, it is possible to calculate $Q$ for each temperature, and to then use this to explain the observed temperature dependence.

At each temperature, the values of $Q$ and $T$ were used to calculate values of $K_8$ and $k_s$ according to equations (3) and (8). From $K_8$, the concentration of $M_8$ was calculated for each temperature, and this combined with the calculated value of $k_s$ to provide a value of $d\bar{M}_w/dt$ using equation (7). This function is plotted in fig. 6 along with the experimentally-determined points. The calculation of $d\bar{M}_w/dt$ in this way introduces no new factors into the mechanism. Fig. 6 shows that the calculated function is in good agreement with observation. The fit is best at the higher end of the temperature range, and the calculated and experimental functions differ at the lowest temperatures used in that the predicted rates are slightly higher than those observed. The generally good fit of the calculated function to the experimental observations suggests that the theory that the reaction is controlled completely by the charge on the protein–$Ca^{2+}$ complex is valid over the whole of the temperature range studied, and that no other factors need be included in defining the mechanism of the aggregation.

## 4. Discussion

From the preceding sections, an overall mechanism for the $\alpha_{s1}$-casein precipitation in the presence of $Ca^{2+}$ may be summarized as follows. Binding of $Ca^{2+}$ to the protein is rapid, and leads to the formation of $\alpha_{s1}$-casein–$Ca^{2+}$ complexes, which contain numbers of bound $Ca^{2+}$ which depend upon the concentrations of casein and $Ca^{2+}$ in the solution, and on the binding constants, which are temperature-dependent. Binding of $Ca^{2+}$ reduces the charge on the $\alpha_{s1}$-casein molecules thereby lowering the repulsive energy barrier between them, allowing an equilibrium between monomers and octamers of $\alpha_{s1}$-casein–$Ca^{2+}$ to become established. The extent of octamerization is dependent on an equilibrium constant which is dependent on the monomer charge. Octamers then take part in an infinite self-association by a Smoluchowski-type process, whose rate-constant is also charge-dependent. Since the monomers themselves are charged, the octamers must also carry a charge on their surface, although some of the original charge may be buried when octamers are formed.

This mechanism accounts for the observed behaviour of $\alpha_{s1}$-casein–$Ca^{2+}$ complexes, in terms of both equilibrium and kinetic properties. It highlights the

critical function of calcium-ion binding, as the major regulatory factor in the process of this aggregation. In such a case, it is to be expected that other divalent cations should precipitate $\alpha_{s1}$-casein according to their binding to the casein. Both $Mg^{2+}$ and $Sr^{2+}$ bind to $\alpha_{s1}$-casein [20] and precipitate the protein, both requiring higher concentrations than $Ca^{2+}$. $Sr^{2+}$ binds less readily than $Ca^{2+}$ and therefore should precipitate the protein less readily. Information on $Mg^{2+}$ binding is contradictory: according to Dickson and Perkins [20], $Mg^{2+}$ binds more strongly than $Ca^{2+}$, but Kaminogawa, Koide and Yamauchi [21] measured the opposite, namely that $Mg^{2+}$ binds considerably less to $\alpha_{s1}$-casein than does $Ca^{2+}$. This discrepancy can only be resolved by further investigation.

The demonstration that ion-binding and charge phenomena alone can explain the temperature-dependence of the $\alpha_{s1}$-casein precipitation may be of importance in the understanding of other precipitation reactions involving caseins. These reactions, such as the heat induced coagulation of milk, appear to have uniformly high apparent activation energies [7]. It is not unlikely that these may be caused simply by the type of effect which is described here by the redistribution of $Ca^{2+}$-binding as a function of temperature. Overall charge of casein micelles has been identified as having a considerable effect upon their stability towards aggregation [22,23] and, although $\alpha_{s1}$-casein forms only a part of the casein micelle, it is the largest fraction of casein in the complex. $\beta$-casein behaves essentially similar to $\alpha_{s1}$-casein (Dalgleish and Paterson, unpublished observations), so that the behaviour of some 80% of the casein involved in the micelle can be described simply in terms of charge interactions.

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