STUDIES ON THE PROPERTIES OF CASEIN FRACITONATED ON DEAE-CELLULOSE (II)

By

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INTRODUCTION

The solubility of protein in water varies within wide limits. Some proteins dissolve in salt-free water, others dissolve only in the presence of certain concentrations of salts. This different behavior of protein has been chosen as a basis for a classification of protein. Particularly the effect of various factors on the solubility of protein in salt solution has been discussed by a number of authors and it is well known that the solubility of proteins depends to a great extent on the concentration of salt present in the solution, and salting-out by high concentration of ammonium sulfate has been used as a classical method of protein separation, on the assumption that the different proteins are precipitated at different levels of fixed ammonium sulfate concentration. On the other hand, the salting-out curve was studied by DERRIEN[2,3] to analyse the properties of protein, and the theoretical basis of the method was reviewed by DIXON and WEBB[4], particularly as applied to the isolation of enzymes. In this paper the method used was to investigate the properties of caseins fractionated on DEAE-cellulose and the effect of heat on the fractionated caseins.

MATERIALS AND METHOD

Materials: The milk was obtained from the Hokkaido University farm and kept at 2°C to 5°C immediately after milking. It was skimmed and cooled at 2°C.

Casein was precipitated from the skim milk which was acidified to pH 4.6 with 1 N HCl. The precipitated casein was dispersed in distilled water of twice the original volume of skim milk. Then it was dissolved with 0.5 N NaOH and precipitated with 1 N HCl again. This procedure was repeated

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seven times. The washed casein was treated with 50% ethanol and then dried with ethanol and ethyl ether. All inorganic and organic salts were commercial products of the best grade available.

Methods: Heating of casein solution: An adequate sample was put in an ampule, and was sealed. The ampule was placed in a water bath at 80°C and in a oil bath at 100°C and 120°C for 30 minutes.

The chromatographic procedure on the DEAE-cellulose (Diethyl-amino-ethyl-cellulose) was performed according to the method of YAGUCHI et al[12].

Salting-out analysis: The salting-out experiment was performed according to a slightly modified form of the method described by TENOW and SHELLMANN[11]. To every tube, phosphate buffer (pH 7.0 and 0.4 M) and saturated ammonium sulfate solution in varied volumes were added, in order to reach the desired degree of saturation in a final volume of 5 ml. 0.5 ml of 1% casein solution were then added to each tube. The tubes were placed in a cold room (2°C) for 12 hours. Finally the suspensions were filtered through filter paper and the absorption of filtrates was measured at 280 mμ.

Sedimentation analysis was performed by the Spinco model E ultracentrifuge apparatus at 56,100 rpm and 30°C.

RESULTS AND DISCUSSION

The properties of fractionated caseins: According to the method of YAGUCHI and TARASSUK[12], casein was fractionated on DEAE-cellulose column.

TABLE 1 Composition of elution buffer and electropholetical properties of fracrionated casein

NaCl concentration of elution buffer (M)	Name of fractionated casein*	Electropholetical properties of fractionated casein**
0.225	A	β-Casein
0.250	B	β-Casein
0.275	C	β- and α-Casein
0.300	D	β- and α-Casein
0.325	E	β- and α-Casein
0.350	F	α-Casein
0.500	G	α-Casein
1.000	H	

* The fractionated caseins were named alphabeticaly in the order of their appearance.

** Electropholetical properties of fractionated caseins were exermined in previous paper[9].

As described in the previous paper[9], whole casein was fractionated into 10 components on DEAE-cellulose column in the range of 0.2 M to 1 M NaCl concentration. The composition and pH value of eluting buffer are given in Table 1. The fractionated components were reprecipitated with 1 N HCl, washed twice with distilled water, and dissolved with 0.5 M NaOH. And then 1% casein solution was prepared and used as sample for all experiments in this paper.

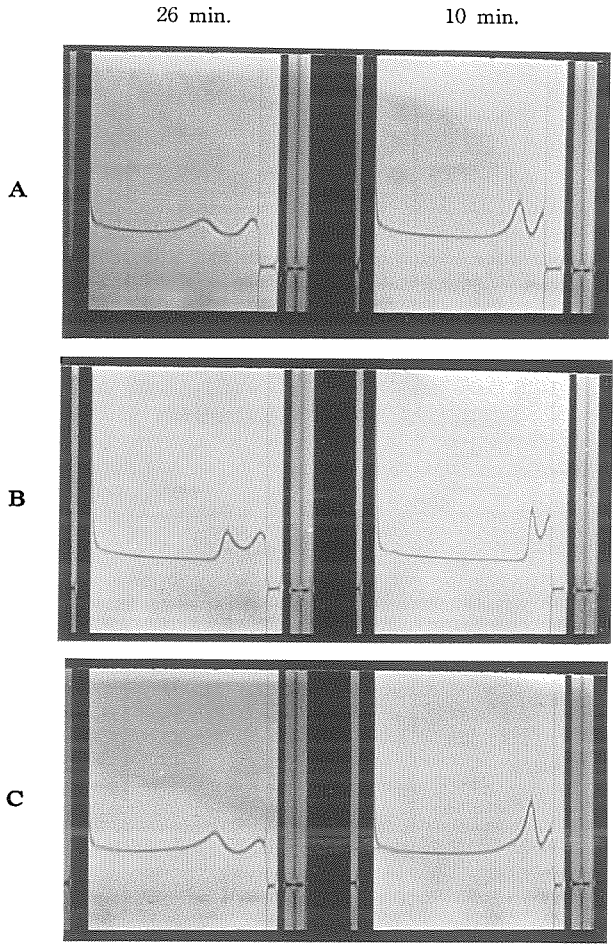
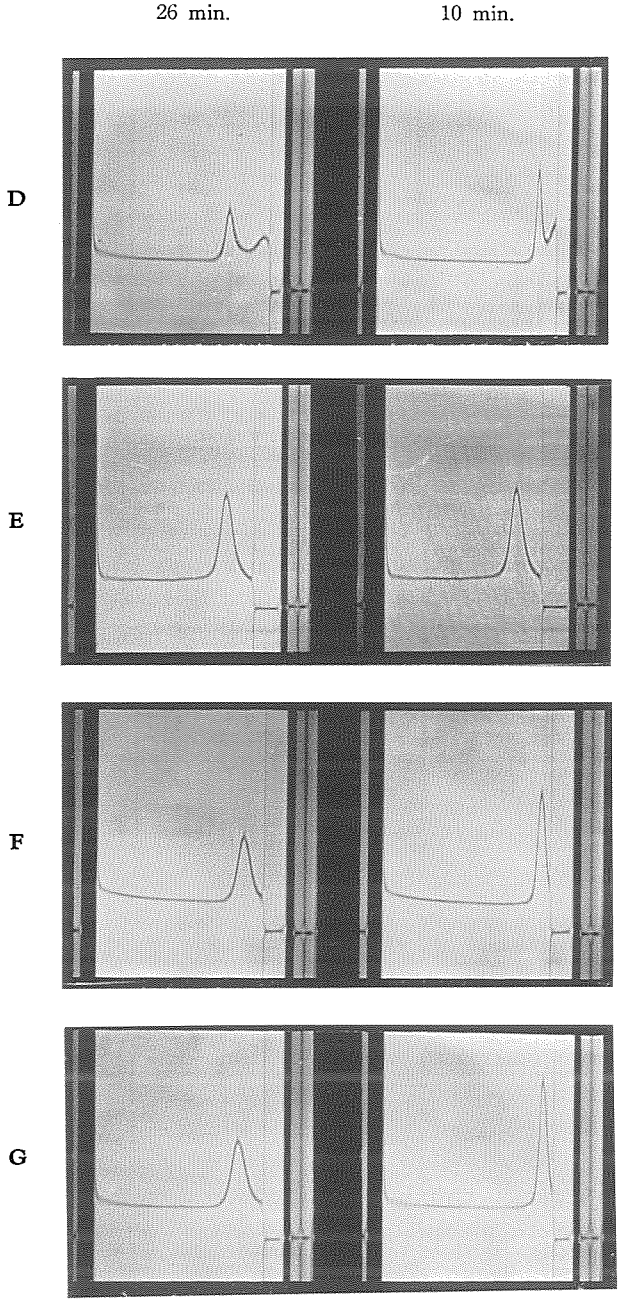


Fig. 1. The sedimentation patterns of fractionated caseins.
Time was measured from the moment when full speed had been reached. The sedimentation proceeded from right to left. The concentration of casein was 0.5% in phosphate buffer solution, pH 6.8, 0.05 M. Alphabetical letter indicated the name of the fractionated casein.


26 min.
10 min.
D
E
F
G

Sedimentation analysis of fractionated caseins was attempted and its results were indicated in Fig. 1. Proteins were classified into two groups ; that is, the one group which showed electrophoretically only α-casein peak was sedimented with a single peak, but the other group containing electrophoretically α- and β-casein complex or β-casein showed two peaks. The component of casein belonging to the first group was electrophoretically and sedimentically homogeneous. This fact indicated the probability that whole casein can be fractionated and purified on DEAE-cellulose column.

The absorption curves of fractionated caseins were studied in respect to ultraviolet wave length. As shown in Fig. 2, all fractionated casein solutions had the maximum absorption value at 280 mμ but there were significant differences in the absorption value. This phenomenon can be explained by the fact that all proteins absorb strongly ultraviolet light at about 280 mμ and the absorption at 280 mμ is caused by the aromatic residues of tryptophane, tyrosine and phenylalanine. According to GORDON *et al.*[5], α-casein and β-casein differed in their contents and the kind of the amino acids and the observed differences were most remarkable in the amount of proline, tryptophane and tyrosine.

The salting-out curve of whole casein and fractionated caseins: The salting-out curve of whole casein is shown in Fig. 4. With about 30% saturated ammonium sulfate, casein produced turbidity at first, began to be precipitated clearly with about 40% saturated ammonium sulfate and was precipitated entirely with 45% saturated ammonium sulfate. The salting-out patterns of caseins fractionated on DEAE-cellulose are indicated in Fig. 3. From those results it is clear that the fractionated caseins should be classified into two groups according to salting-out patterns.

Fractionated caseins of one group were precipitated clearly with about 15% saturated ammonium sulafte and precipitated almost entirely at about 20% ammonium sulfate solution. According to the electrophoretic analysis, the caseins consist of α-casein. On the other hand, caseins of the other group showed a remarkable change at about 40% saturated ammonium sulfate and were precipitated entirely at about 45% satnrated ammonium sulfate. Electrophoretically, those caseins were β-casein or a mixture of α- or β-casein.

According to Derrien[2], when a protein mixture is progressively precipitated in a series of media with increasing salt concentration, an equilibrium will be attained between precipitated and dissolved proteins in each medium after a certain time, and the amount of protein remaining in solution will depend upon the concentration and the nature of proteins, the pH and temperature of the medium.

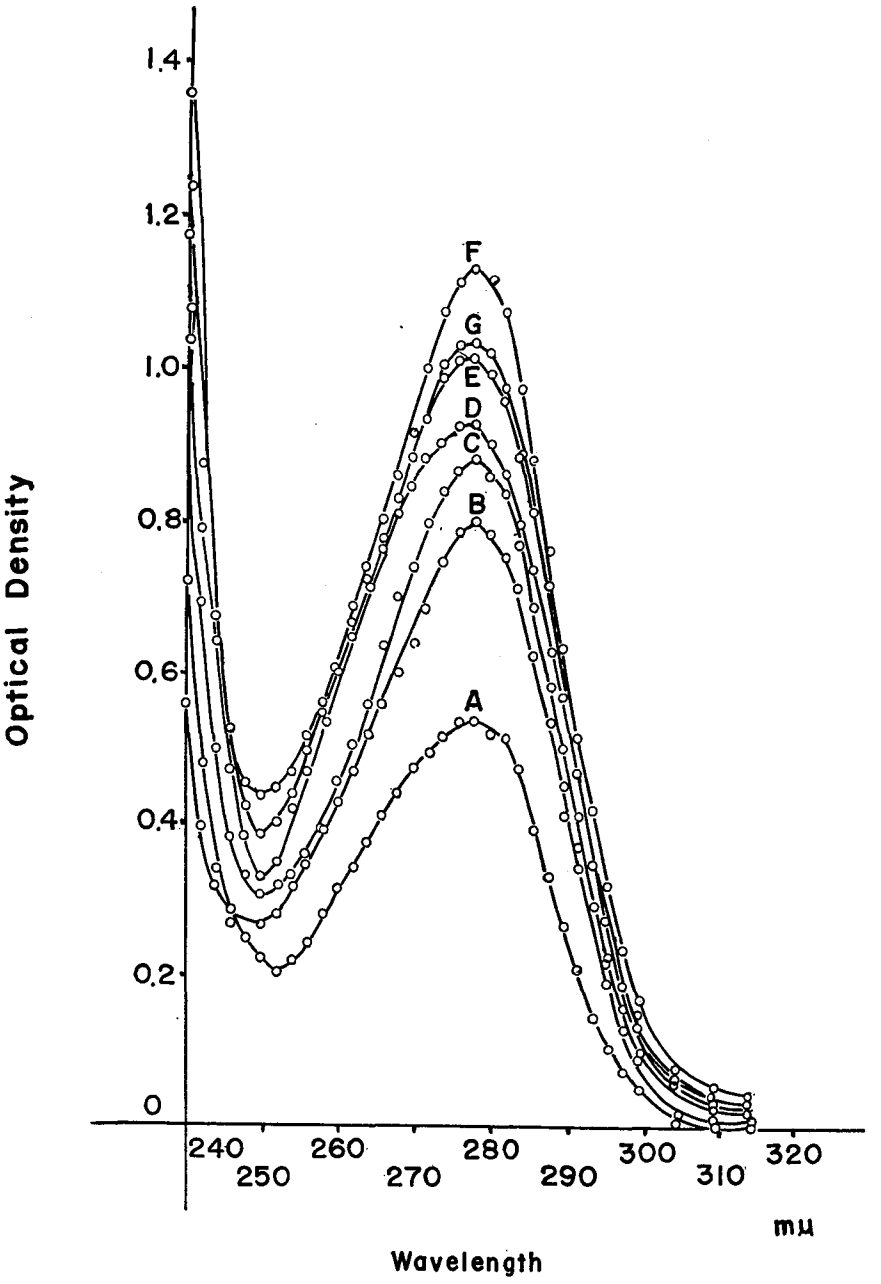


Fig. 2. Ultraviolet absorbtion curves of fractionated caseins.

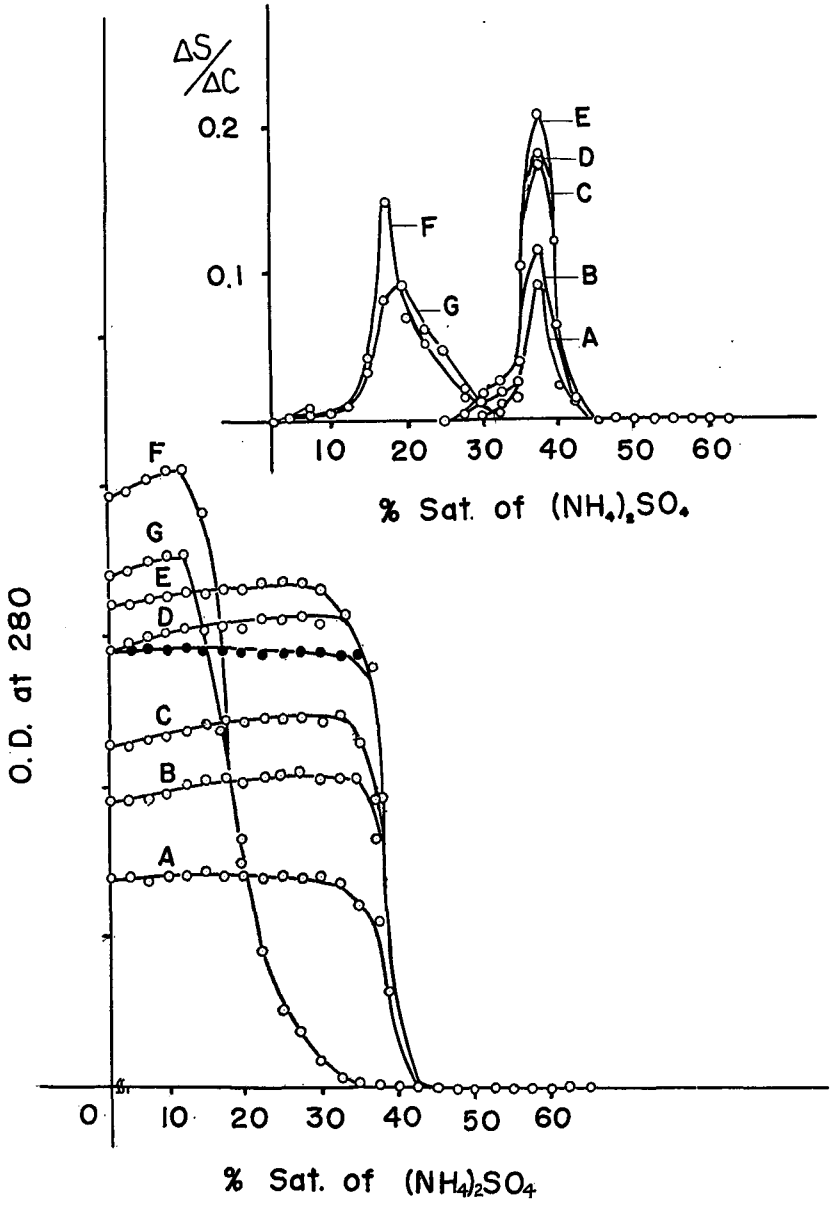


Fig. 3. Salting-out curves of fractionated caseins.

Derived curve $\left(\frac{\Delta s}{\Delta c}\right)$; Δs = absorption value for each increment Δc of the salt concentration.

It is well known that the solubility of protein in salt solution is modified with many factors. The salting-out effect of high concentration salts is evidently due to a competition between the salt and the protein for the molecule of the solvent. The solubility of the proteins depends mainly on the nature of the chemical groups which form the surface of the protein molecule, and particularly on the distribution of ionic and nonpolar groups between the sfurface and the interior of protein molecule.

From the reason mentioned above, it can be considered that the differences in salting-out curves of fractionated caseins may depend upon the differences in the chemical formula, the condition and the shape of their molecules. This view is supported by the following facts: GORDON *et al.*[5] have published complete amino acid analysis of α- and β-caseins and calculated that α-casein contained 548 polar and 341 nonpolar groups per 10^5 g., and β-casein 465 polar and 442 nonpolar groups per 10^5 g. HIPP *et al.*[6] reported that the compositional differences are in line with the electrophoretic mobilities and ethanol-water solubilities. From the titration curves, HIPP *et al.*[7] have determined the acid- and base-binding capacities of the principal components of casein, namely α-, β- and γ-casein, and showed a marked difference in the properties of these proteins. Moreover, many authors reported differences in molecular weight, isoelectric point and electrophoretic mobility between α- and β-casein. In addition, as observed in Fig. 1, and Table 1, each casein fractionated on DEAE-cellulose column had different properties.

The effect of heat on the salting-out curves of whole casein and fractionated caseins: It is well known that the solubility of protein changes greatly on denaturation and the change of solubility has been used as a criterion for the denaturation of proteins. As shown in Fig. 4 and 5 the salting-out curves of caseins change with heat treatment. The solubility of whole casein in ammonium sulfate solutions decreased with heat. But the fracionated caseins behaved differently. As described above, the fractionated caseins were classified into two groups according to the differrences in salting-out. The group which changed remarkably at about 40% saturated ammonium sulfate showed a decrease in solubility in ammonium sulfate solution by heating, however the other group which showed a remarkable change with 20% saturated ammonium sulfate had a tendency to increase in solubility in ammonium sulfate solution. It is not easy to explain why such a difference causes. Generally it is considered that the decrease in solubility is one of the most important changes occurring with the denaturation of most proteins. But SEGAL[10] described that under certain condition denaturing might even increase the solubility of protein.

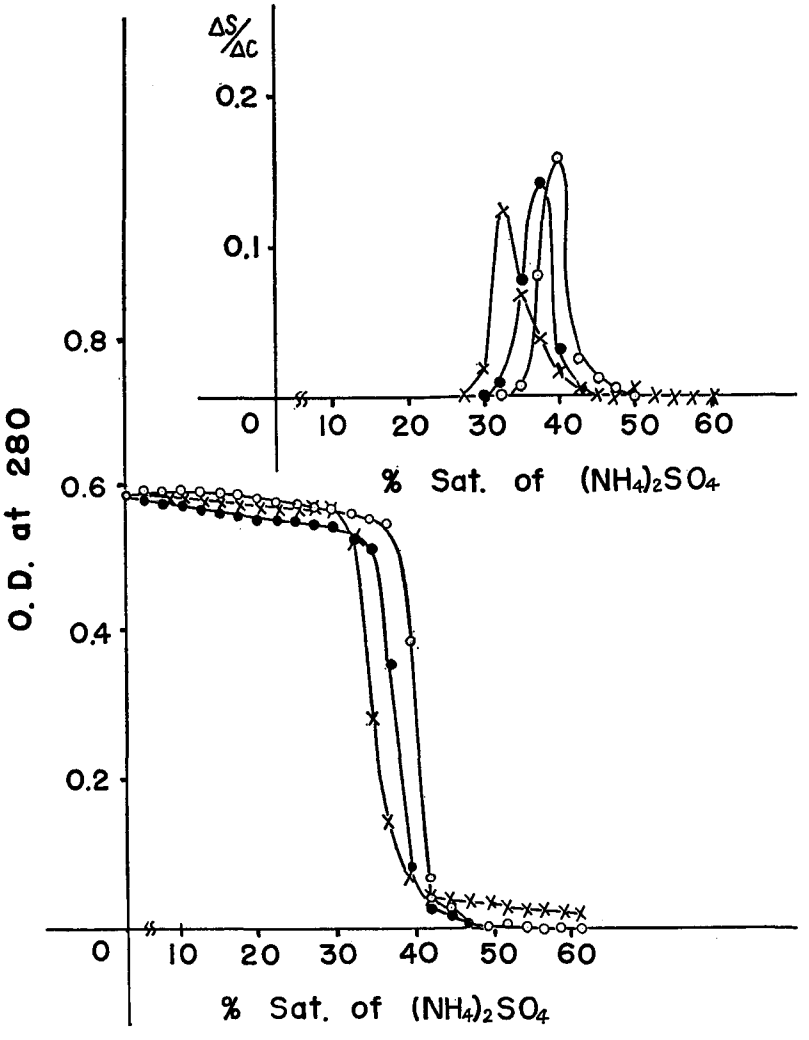


Fig. 4. The effect of heat on salting-out curve of whole casein. Derived curved $\left(\frac{\Delta s}{\Delta c}\right)$; Δs=absorption value for each increment Δc of the salt concentration.

—○—: control
—●—: casein solution heated at 100°C.
—×—: casein solution heated at 120°C.

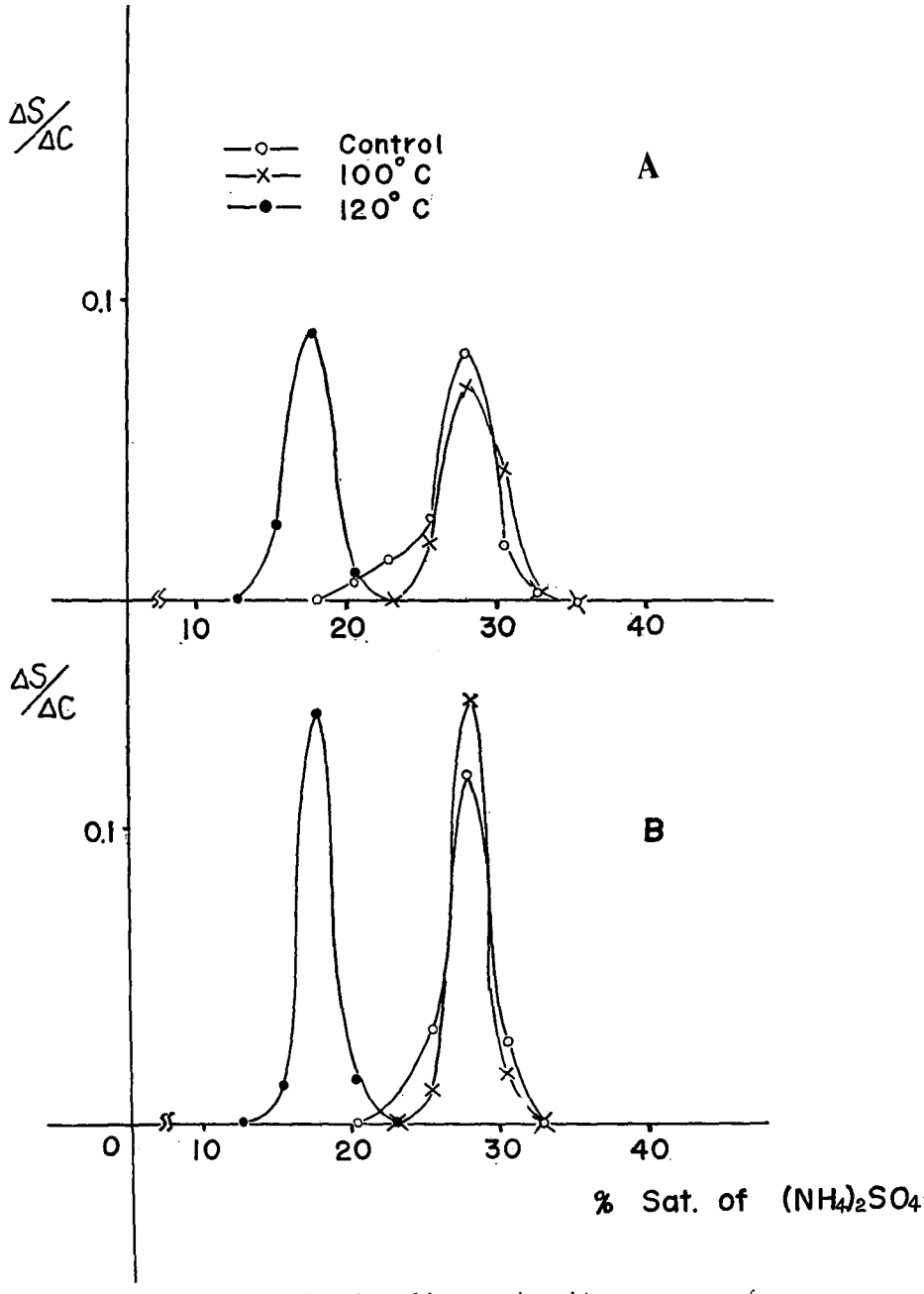
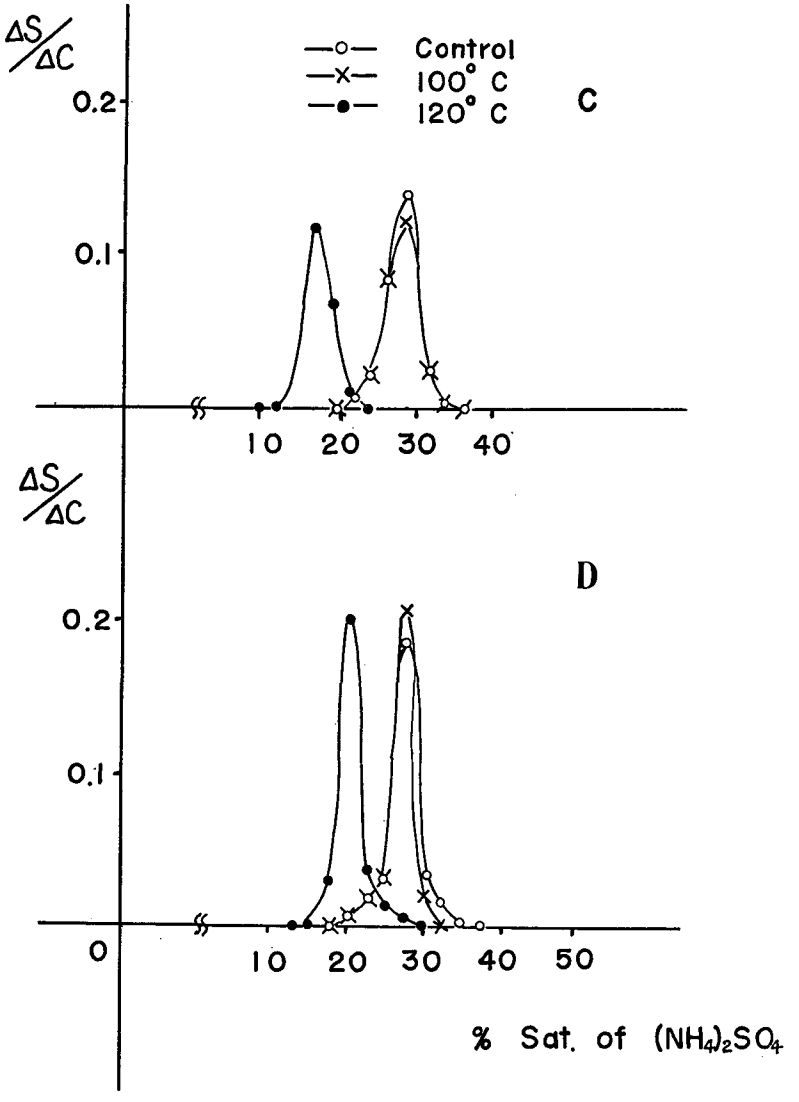
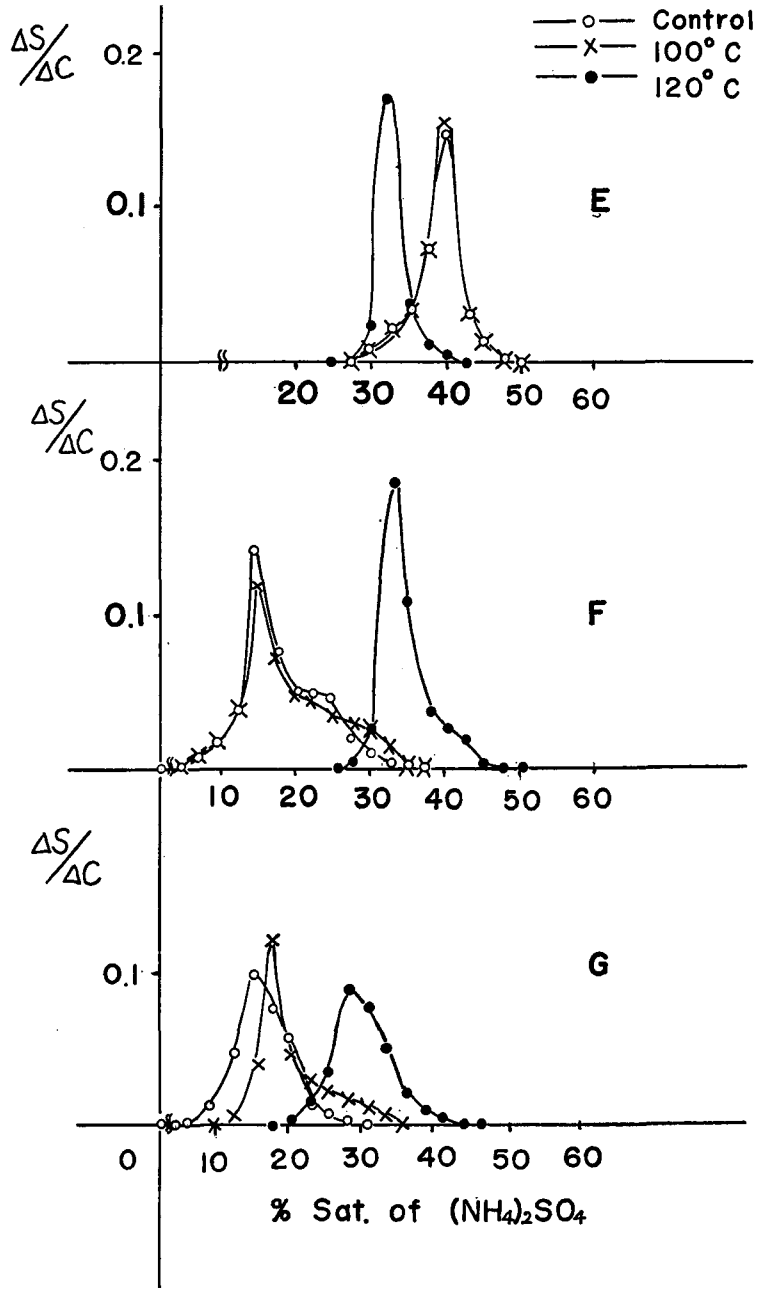


Fig. 5. The effect of heat on the salting-out curves of fractionated caseins.



Derived curve $\left(\frac{\Delta s}{\Delta c}\right)$; Δs=absorption value for each increments Δc of the salt concentration.


Control
100° C
120° C
ΔS/ΔC
0.2
0.1
E
20 30 40 50 60
ΔS/ΔC
0.2
0.1
F
10 20 30 40 50 60
ΔS/ΔC
0.1
G
0 10 20 30 40 50 60
% Sat. of (NH4)2SO4

The effect of various factors on the solubility of a protein in salt solution is very complicated. However it can be considered that the solubility of proteins depends mainly on the nature of the groups which form the surface of the large particles, and particularly on the distribution of ionic and non-polar groups between the surface and the interior of the protein molecule.

Casein is often thought to be stable, and no changes seem to occur in its chemical and physical properties in the course of heat treatment which denatured serum proteins, but casein is denaturated markedly by heating above 100°C. HWOAT and WRIGHT[8] reported that casin solutions lost ester phosphate and some nitrogen when heated to 120°C. BELEC and JENNESS[1] mentioned that casein solutions heated at 100°C and 140°C progressively released phosphate. YUSA[14] found that when milk was heated at 125°C, casein particles tend to aggregate. YAMAUCHI and TSUGO[13] pointed out that heating at 100°C for 30 minutes produced no marked change in the electrophoretic pattern of casein, while heating at 120°C caused electrophoretical changes. Thus the nature of the chemical groups which form the surface of casein molecule was considerably changed with heat treatment, and the changes occured may influence upon the solubility of casein in salt solution.

SUMMARY

1. With about 30% saturated ammonium sulfate solution whole casein produced turbidity at first and then was precipitated entirely at 45% saturated ammonium sulfate.

2. The caseins fractionated on DEAE-cellulose column were classified into two groups according to the differeneses in salting-out patterns, that is, one group of fractionated caseins was precipitated entirly with about 20% saturated ammonium sulfate and the other group was precipitated with about 40% saturated ammonium sulfate.

3. The solubility of whole casein in ammonium sulfate solution decreased when it was heated above 100°C, but the fractionated casein on DEAE-cellulose showed different behaviors; one group which consisted of β-casein or α- and β-casein mixture electropholetically tended to decrease in solubility in ammonium sulfate solution and the other grop which consisted of α-casein had atendency to increase in solubility after heat treatment.

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