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AGGREGATION OF BOVINE SERUM ALBUMIN UNDER HIGH PRESSURE

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Some works have been done on the effect of high pressure on solutions of serum albumin.[1)–7)] Changes in the properties of serum albumin caused by the pressure treatment were found by turbidity measurement,[1)2)7)] flow birefringence[1)4)5)] and by Tiselius electrophoresis.[3)]

The authors analyzed solutions of bovine serum albumin (BSA) treated under high pressure by an ultracentrifuge and acrylamide gel electrophoresis.

A 1% solution was prepared at pH 8.9. Pressures of 1000–3000 kg/cm^2 were applied at 25–40°C for 15–60 minutes. Sedimentation experiments were conducted at 20°C and at 59,780 rpm, using a Spinco Model E ultracentrifuge. Gel electrophoresis was conducted at pH 8.9. After electrophoresis, the gel was stained with Amido-black 10B.

Solutions after the pressure treatment were quite transparent, and were not different visually from the native BSA solution. There were three boundaries in the ultracentrifuge pattern (Fig. 1).

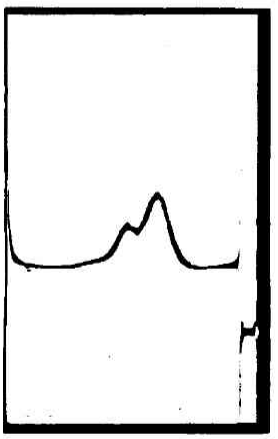

Fig. 1 Ultracentrifuge pattern of BSA treated under 3000 kg/cm^2 for 60 min. at 35°C. Sedimentation from right to left; 4,800 sec. after reaching full speed (59,780 rpm.)

The sedimentation coefficients S and the areas covered with each boundary are given in the Table below.

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1) M. Macheboeuf, J. Basset, E. Barbu, M. Lesaget and G. Nunez, *Compt. rend. Soc. Biol.*, **144**, 962, 964 (1950)
2) V. S. Tongur and V. I. Kasatochkin, *C. A.*, **47**, 12438e
3) E. Barbu, M. Macheboeuf, P. Reberotte and P. Slizewicz, *Bull. Soc. Chim. biol.*, **34**, 724 (1952)
4) E. Barbu, J. Basset and M. Joly, *ibid.*, **36**, 323 (1954)
5) E. Barbu, J. Basset and M. Macheboeuf, *C. A.*, **48**, 9433i
6) C. Suzuki, K. Kitamura, K. Suzuki and J. Osugi, *This Journal*, **32**, 30 (1963)
7) C. Suzuki and K. Suzuki, *Arch. Biochem. Biophys.*, **101**, 225 (1963)

	Sed. coeff.	Area covered
Slowest boundary	4.0 *S*	65%
Middle boundary	5.0	30
Fastest boundary	6.8	5

It is concluded from the *S* value that the slowest boundary is the native BSA, the middle boundary is a dimerized BSA,[8] and the fastest boundary is higher aggregates.

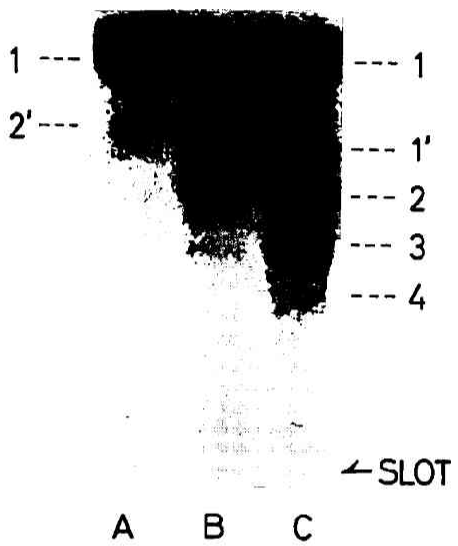

Fig. 2 Patterns of acrylamide gel electrophoresis of BSA. Gel concentration was 5%.
A. Native BSA
B. 2000kg/cm² was applied 45 min. at 35°C
C. 3000 kg/cm² was applied 60 min. at 35°C

BSA solutions after pressure treatment were separated into a few, clear, discrete zones by gel electrophoresis. The typical patterns are shown in Fig. 2. In the patterns A, B, and C, the zone 1 is native BSA. The zone 2′ is the dimer-impurity contained in the original sample. When the pressure was 2000kg/cm², there were 4 zones; when it was 3000kg/cm², there were 5 zones. The separation of the zones was obvious and there was no tailing in the pattern. In addition, the sample slot was quite transparent indicating that the gross particles which cannot penetrate the gel pore were not formed.

Solutions of BSA treated by high pressure were analyzed changing the gel concentration.[9] The number of zones did not change due to the change in gel concentration. Taking the migration of the zone 1 as a standard, the migration ratio of each component was calculated. Fig. 3 was obtained by plotting the migration ratios against gel concentration. As is seen, a horizontal line was obtained for the zone 1′, and the sloping lines were obtained for zones 2, 3 and 4. These results indicate that the component in the zone 1′ is monomeric albumin and that components in the zones 2 to 4 are polymeric. This is an interpretation similar to that of Raymond.[9]

Combining the results by ultracentrifuge with those by gel electrophoresis, the following are

8) P. Bro, S. J. Singer and J. M. Sturtevant, *J. Am. Chem. Soc.*, **80**, 389 (1958)
9) S. Raymond and M. Nakamichi, *Anal. Biochem.*, 7, 225 (1964)

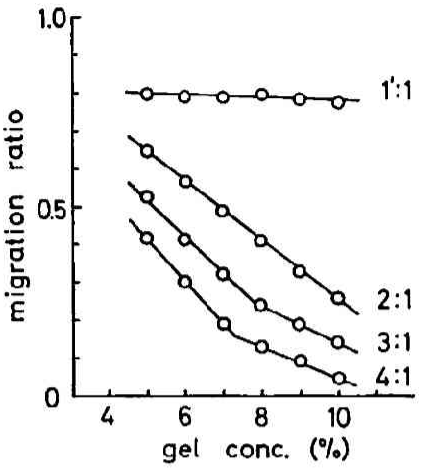

Fig. 3 Migration ratio *vs.* gel concentration
Migration of zone 1 was taken as a standard.

concluded. While in gel electrophoresis the zones 1 and 1′ are separated, these two components are included in the slowest boundary of ultracentrifuge pattern. This indicates that the component 1′ is monomeric albumin which differs slightly from the native BSA in electric charge and/or shape. It is confirmed that the component 2 is dimerized albumin, and that components 3 and 4 might be higher aggregates. It seems likely that the first effect of high pressure on BSA is to dimerize it. A possibility is that the component 1′ is an intermediate of dimerization, and the dimerization proceeds from 1 to 2 *via* 1′.

The facts that the number of zone changes and that the intensity of staining of each zone changes with the condition of pressure treatment, give some insight into a mechanism by which BSA aggregates under high pressure. A study on this line is now going on, and the results which would be obtained, with the apparatus used, will be published elsewhere in the future.

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