Interaction of an alkali stable polysaccharide from cell surface of *Staphylococci* with human fibrinogen

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Summary. The effects of enzymatic digestions and physicochemical treatments on an alkali stable polysaccharide, which polymerize fibrinogen, extracted from a strain of *Staphylococcus epidermidis*, were observed in the demonstration of gel formation of fibrinogen.

The blood clotting mechanisms is generally understood to be a collaboration of enzymatic reactions involving several factors resulting in a structural change of fibrinogen. Recently, however, a combination of a sialic acid derivative[1], a carbohydrate[2], and a protein clumping factor[3,4] have been considered to interact directly with fibrinogen resulting in paracoagulation. A special case concerns the staphylococcal blood clotting capacity of coagulase. This substance, called staphylothrombin, is produced by strains of *S. aureus* and reacts with fibrinogen to form a fibrin clot[5,6]. On the other hand, recently we noted a cell surface substance of staphylococcal strains resulting compact-colony formation in serum or fibrinogen soft agar medium. This substance was an alkali stable polysaccharide, different from coagulase, clumping factor and protein A and was designated as the compact-colony forming active substance (CCFAS)[7]. In our more recent contribution[8], it was reported that fibrinogen polymerization or gel formation could be caused by this alkali stable polysaccharide (ASP). In these experiments, using the ASP obtained from a strain of *S. epidermidis*, effects of enzymatic digestions and physicochemical treatments upon fibrinogen causing gel formation were observed by determining increasing turbidity of fibrinogen.

Material and methods. For the extraction and purification of the ASP[11], the staphylococcal strain SMU 98 was used. The organisms were negative for coagulase, mannitol fermentation, acid phosphatase and for clumping factor reaction and positive for deoxyribonuclease determined by the method described elsewhere[9]. With these biological properties, the strain was identified as *S. epidermidis* according to the description of Zierdt et al[10]. Since the ASP was an associated polysaccharide[11], prior to mixing with fibrinogen it was treated with enzymes such as glucose oxidase, galactose oxidase, and N-acetylglucosaminidase (Biochemical Co., Ltd, Tokyo). Namely, to 1.0 mg ASP dissolved in M/15 phosphate buffer, pH 7.4, 0.1 mg glucose oxidase, α-galactosidase, β-galactosidase, galactose oxidase, β-N-acetylglucosaminidase or mixed glucosidases was added and maintained at 37 °C for 6 h. The solution was deproteonized, dialyzed against the same buffered solution, and finally purified by the method described previously[11]. Then, it was dissolved in 0.1 ml of the same buffered solution and incubated at 37 °C. Gel formation time of the

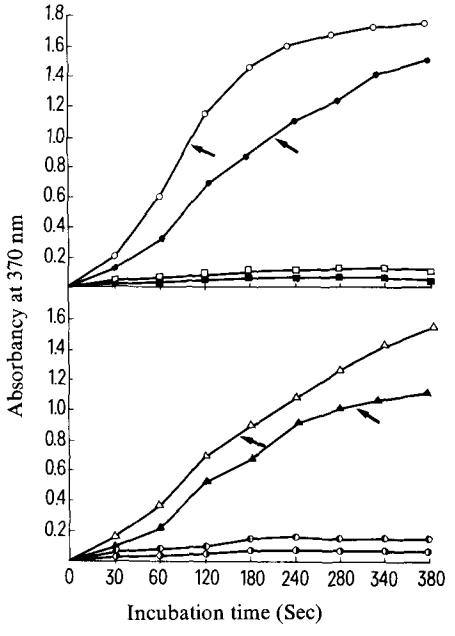

Fig. 1. Effect of various enzymes on the change in turbidity of fibrinogen by the addition of the alkali stable polysaccharide extracted from a strain of *Staphylococcus epidermidis*. ◑——◑, ●——●, □——□, ■——■, △——△, ▲——▲, ◐——◐, and ○——○ indicate the turbidities of fibrinogen alone, fibrinogen combined with the alkali stable polysaccharide treated with α-galactosidase, β-galactosidase, glucosidase, glucose oxidase, β-N-acetylglucosaminidase, mixed-glucosidase and untreated, respectively. Arrow means the time of gel formation.

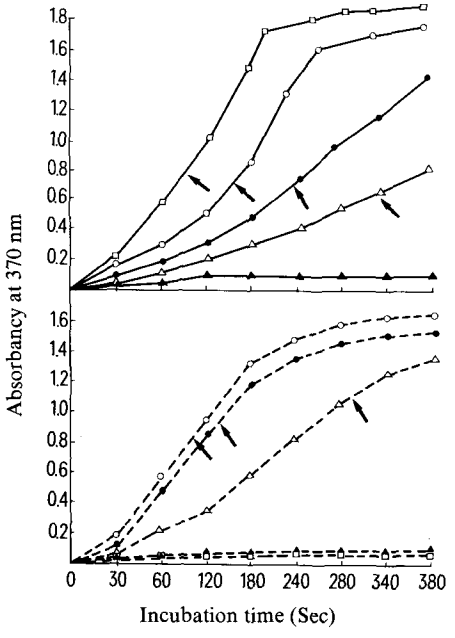

Fig. 2. Effects of periodate oxidation, various pH and heating on the change of turbidity of fibrinogen by the addition of alkali stable polysaccharide extracted from strain of *Staphylococcus epidermidis*. ○——○, ●——●, △——△, ▲——▲, ○ ----- ○, ●----- ●, □ ----- □, △ ----- △, ▲ ----- ▲, and □——□ indicate the turbidities of fibrinogen combined with the alkali stable polysaccharide treated with KIO_4 for 5, 10, 15, 20 h and pH 6.0, 8.0, 10.0 and heated at 120 °C for 10, 30 min and untreated, respectively. Arrow means the time of gel formation.

fibrinogen was then obtained by recording the increase in the turbidity of fibrinogen polymerization by nephelometry at 370 nm according to the method of Michael et al.[12]. To elucidate further the polysaccharide nature of the ASP, it was treated with 0.01 M KIO_4 in tris-hydrochloride buffer, pH 8.4, at 27 °C for 5, 10, 15 and 20 h. Heat stability of the ASP was tested by maintaining at 120 °C for 10 and 30 min in tris-hydrochloride buffer, pH 8.4. For the examination of the effect of different pHs on its biological stability, it was dissolved in phosphate buffer at pH 6.0, 8.0 or 10.0. These were determined by the gel formation time as mentioned above.

Results. Normal gel formation time of fibrinogen mixed with intact ASP alone was 90 sec whereas it was 150, 230 and 330 sec when it was pretreated with glucose oxidase, α-galactosidase and β-N-acetylglucosaminidase, respectively. However, no gel formation was observed after treatment of ASP with galactose oxidase, β-galactosidase or mixed glucosidases. The increase of turbidity for fibrinogen polymerization matched the gel formation time as shown in figure 1. With treatment by periodate oxidation for 5 h, the ASP prolonged the gel formation capacity. With the same treatment for 20 h, gel formation was completely destroyed. When the ASP was heated at 120 °C for 10 min, gel formation time was prolonged to 320 sec; however, this activity was completely destroyed by heating for 30 min. Gel formation was lost below pH 6.0, but was quite stable between pH 8.0 and 10.0 as shown in figure 2. Further, similar results were observed when prothrombin-free plasma was used in place of fibrinogen.

Discussion. In a previous paper[11] it was noted that the ASP, designating as the CCFAS, was capable of reacting either with fibrinogen or fibrinogen degradation products resulting compact-colony formation of *S. aureus* strains in fibrinogen or serum-soft agar. The substance was totally different from protein A, coagulase and clumping factor, as mentioned above, and contained neither sialic acid nor any common enzymes including the proteolytic enzymes. Thus the ASP was considered to be the 3rd staphylococcal substance related to blood clotting in addition to coagulase and the clumping factor.

In these experiments, the ASP obtained from *S. epidermidis* was sensitive in its biological activity to both galactose oxidase and β-galactosidase, but was resistant to β-N-acetylglucosaminidase. These properties suggest that the digestion of the galactose moiety in the ASP could be caused by 2 former enzymes. Also, it was suggested that the ASP may convert fibrinogen to fibrin by direct action. In this case, the carbohydrate moiety of this substance, especially galactose, would be closely related to fibrinogen polymerization in an alkaline condition. This alkaline stability of the ASP would represent a biological advantage in normal alkaline tissue fluid. Further investigation concerning the relationship between fibrinogen polymerization by the ASP and other blood clotting substances are in progress in our laboratory.

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An antibody to low density lipoprotein in diabetics

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Summary. The serum of 21/180 unselected diabetics contains a low titre antibody to human low density lipoprotein. A similar antibody was found in 10/88 persons with tuberculosis.

Iso-antibodies against low density lipoprotein (LDL) or high density lipoprotein (HDL) are found in patients who have been transfused with blood or plasma repeatedly[1], in occasional persons with myeloma[2], and as a cause of hypolipidaemia in rheumatoid arthritis and cancer[3]. Some years ago Beaumont, Jacotot and Beaumont[4] found antibodies to LDL in patients with advanced ischaemic heart disease. Although they then suggested that the antibody might be a cause of atherosclerosis, the finding could be a secondary phenomenon. This report concerns the finding of anti-LDL antibodies in patients with either diabetes mellitus or pulmonary tuberculosis.

Methods. Sera were collected from unselected patients with diabetes, pulmonary tuberculosis or nephritis. Specimens sent to the biochemical laboratory were used as controls. The sera were decomplemented by heating at 55 °C for 30 min and were then adsorbed with sheep red cells. Agglutination tests were performed with sheep red cells coated with chromic chloride[5] to which LDL antigen was attached. This LDL was obtained from human serum by manganese chloride-heparin precipitation[6]. After dialysis separation of the LDL was achieved by ultra-centrifugation in a sodium chloride density tube at 105,000 × g for 18 h. In this way lipoproteins Sf 0–12 were obtained for use as antigen. The preparation was Australia antigen negative.

Results. These are shown in the table according to the haemagglutination titres using doubling dilutions of serum. 40 µg of antigen when added to 0.3 ml serum was found to reduce the antibody titre from $\frac{1}{8}$ to $\frac{1}{2}$.

It will be noted that 11% of diabetics and 11% of patients