Short Communications

On the Preparation and Properties of Sulfitolysed Fibrinogen and Fibrin

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The possible importance of the sulfur-containing groups in the different steps of blood coagulation has been discussed for a long time. It was, for example, suggested that the fibrinogen-fibrin conversion consisted of an aggregation of fibrinogen molecules due to the oxidation of their sulfhydryl groups and the formation of intermolecular disulfide bonds¹⁻³. However, when Bagdy *et al.* and Carter and Warner attempted to determine the sulfhydryl groups present in fibrinogen, no such groups were found⁴⁻⁶. Since then we have investigated the presence and number of sulfhydryl groups and disulfide bonds in both fibrinogen and fibrin using several kinds of electrometric titration methods. None of these techniques could reveal any free sulfhydryl groups⁷. The number of disulfide bonds per molecule corresponded closely to the number of half-cystine residues as determined by amino acid analysis after performic acid oxidation⁸. As a further step in the analysis of the function of sulfur-containing groups in the fibrinogen-fibrin conversion, we have studied some properties of fibrinogen and fibrin, which had been treated with disulfide-splitting reagents. A short report on these experiments has already been given by us some time ago⁹. Apart from this no disulfide-modified fibrinogen and fibrin derivatives have been described.

In the present investigation, we have attempted to obtain derivatives of fibrinogen and fibrin in which the sulfur bonds are

quantitatively split by sulfite, but in which there are no other modifications. Some of the properties of these derivatives and the thrombin-induced transformation of the fibrinogen-derivative were also studied. Bovine and human fibrinogen and fibrin, purified according to Blombäck and Blombäck, were used¹⁰. Principally two different methods of sulfitolysis were employed. In most cases we used a slight modification of the method described by Swan¹¹ and by Pechère *et al.*¹² The protein was dissolved in 8 M urea and treated with sodium sulfite and cupric sulfate usually at a pH of 9.0 at room temperature for one hour. In some cases we used the method described by Bailey and Cole¹³. The protein in an 8 M urea solution was intermittently treated with sodium sulfite and sodium tetrathionate at a pH of 7.4 at +38°C. After the sulfitolysis, the protein was separated from the reagents by gel filtration on Sephadex G-50¹⁴ or by dialysis. The number of unchanged disulfide bonds or free sulfhydryl groups was determined by electrometric titration. The yields were calculated from spectrophotometric values¹⁵ and from the weights of the lyophilized material.

The products of sulfitolysis of fibrinogen and fibrin were almost quantitatively recovered. If low molecular weight materials had been formed during sulfitolysis, they should have been lost during isolation by dialysis or gel filtration. This seems not to be the case. For these reasons, the products have been termed S-sulfo-fibrinogen and S-sulfo-fibrin. Some experiments were performed to estimate the size of the derivative molecules. Using Sephadex gels of various molecular sieve properties, no separation of different fractions of the S-sulfo-proteins could be obtained even in 6 M urea solution. Preliminary ultracentrifuge studies showed homogeneity of the substances and a considerable decrease in molecular weight as compared to untreated fibrinogen.

Both the S-sulfo-proteins were, when obtained by dialysis or gel filtration, completely soluble at low concentrations in distilled water. They showed, however, a marked tendency to aggregate when the ionic strength of the solution was increased or its pH was lowered. After lyophilization the products were no longer soluble in water, but dissolved immediately in solutions of, for example, urea or sodium dodecyl sulfate. Solutions of S-sulfo-fibrinogen or S-sulfo-fibrin in distilled water or 0.15 M sodium chloride did not exhibit the precipitability at + 58°C, which is characteristic of untreated fibrinogen.

The S-sulfo-proteins were studied by means of paper and starch gel electrophoresis. The paper electrophoresis pattern varied considerably from one solvent to another. In most urea-containing buffers two to three bands could be distinguished. Whether derived from S-sulfo-fibrinogen or from S-sulfo-fibrin, one or two of the bands showed the same mobility, whereas remaining bands differed according to their source. In all other buffer systems tried, only a single band was demonstrable, even when the buffers contained other reagents, such as sodium dodecyl sulfate or dimethylformamide, capable of splitting hydrogen bonds. Starch gel electrophoresis in 6—7 M urea showed two or three bands, all of which had considerably greater mobility than fibrinogen and fibrin.

The completeness and specificity of the sulfitolytic procedure was controlled. On electrometric titration of the S-sulfo-proteins no remaining sulfhydryl groups or disulfide bonds were found. N-Terminal amino acid analysis, by the phenylisothiocyanate method, showed that the S-sulfo-derivatives had the same N-terminal groups in the same amounts as have the unmodified proteins, which indicates that no peptide bonds were broken during sulfitolysis. The amino acid compositions of the S-sulfo-derivatives seemed to be the same as those of the native proteins.

It was possible to separate different fractions of S-sulfo-fibrinogen and S-sulfo-fibrin on ion exchange Sephadex, diethylaminoethyl- and carboxymethyl-Sephadex. Three components were isolated, which had different electrophoretic mobilities in urea-containing buffers, and which corresponded to the previously observed bands. When recombined the components gave the original pattern.

The possible change in the reaction of the modified fibrinogen in the presence of

thrombin was studied. S-Sulfo-fibrinogen was found to be a substrate for thrombin. However, it did not coagulate in the ordinary sense, but formed a fine precipitate. This occurred about as rapidly as the formation of a native fibrin coagulum, provided that the same conditions of pH, ionic strength and temperature were maintained. Approximately 80 % of the S-sulfo-fibrinogen was precipitable with thrombin. S-Sulfo-fibrinogen was apparently more thermostable than fibrinogen, and the velocity of its reaction with thrombin was unchanged after heating to + 60°C. N-Terminal amino acid analysis indicated that in the presence of thrombin, the peptides were released from S-sulfo-fibrinogen in the same order and at about the same rate as from native fibrinogen. S-Sulfo-fibrin exhibited the same electrophoretic pattern, regardless of whether it had been obtained from S-sulfo-fibrinogen with thrombin or from native fibrin by sulfitolysis.

This work will be described and discussed in detail in the near future.

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